Translated from *Uspekhi Khimii*, 52 138-167 (1983) U.D.C. 547.963.3

Complementation Interactions of Oligonucleotides

N.G.Dolinnaya and E.S.Gromova

The results of studies by NMR, ultraviolet spectroscopy, circular dichroism, and X-ray diffraction of complexes of complementary oligonucleotides containing between two and fifteen monomeric units are surveyed. The review deals with tne structures of the complexes in solution and in the crystalline state and the thermodynamics (using different theoretical models) and kinetics of their formation. The influence of the nucleotide composition and of the sequence and length of the oligomers on the structure and properties of the complexes as well as the biological importance of these systems are discussed. Attention has been devoted to the question of the stability of individual pairs of nucleotide complexes as a function of their nature and position in the oligomer chain.
The bibliography includes 124 references.

CONTENTS

I. Introduction 79
II. Types of oligomeric complexes investigated 80
III. Thermodynamic description of the complex formation by complementary oligonucleotides 80
IV. Characteristic features of the thermodynamics of complex formation by heterogeneous oligonucleotides 84
V. Kinetics of the formation and dissociation of the oligomeric complexes 86
VI. Complementation interactions of dinucleotides 87
VII. The structure of the oligomeric complexes 87

I. INTRODUCTION

The complementary complexes of synthetic poly(oligo)nucleotides are widely used as models of fragments of nucleic acids (NA) for the solution of many problems in molecular biology. The thermodynamic and kinetic aspects of the formation of complementary pairs, necessary for the understanding of any processes involving the breakdown and formation of double helices, are investigated with their aid. The role of nucleotide complexes has been increasing in the study of the protein–nucleic acid interactions, of the mechanism of the action of enzymes in nucleic acid metabolism, etc. The complexes of oligodeoxyribonucleotides, containing sequences which are split by restriction enzymes, recently found a new application—for the construction of recombinant DNA molecules. They serve as linkers in the addition of a natural or synthetic DNA molecule to the cloning vector or as adaptors with the aid of which additional restriction sites as well as DNA fragments, for example the initiating and terminating codons, the Shine–Delgarno sequence, etc. are built into the recombinant molecules.

Previously homopolynucleotides and polymers with repeating di(tri)nucleotide sequences mainly served as models of nucleic acids. Recently, in connection with considerable advances in synthetic nucleotide chemistry and development of methods of genetic engineering, complexes of oligo(poly)nucleotides with heterogeneous sequences have become available. Complexes of short nucleotides are used on a particularly wide scale. They make it possible to alter specifically the length of double helix sections and to vary the nucleotide sequence. Since short oligonucleotide complexes readily dissociate in aqueous buffer solutions at moderate temperatures, it is convenient to investigate with their aid any properties associated with the breakdown of double helix structures and the thermodynamic description of such systems can be based on simpler models. Oligomeric systems make it possible to investigate the influence of the length of the double helix section and of the nucleotide sequence on the thermodynamic and kinetic parameters of the complex formation as well as the optical properties of the double helices. Oligomeric systems are the most convenient objects for the elucidation of the kinetics of the formation of complementary complexes and of the influence of "error" bases on the stability and structure of the double helices.

Several reviews, constituting chapters of monographs and textbooks in English, have been devoted to the problems of the interaction of complementary oligonucleotides[1–4]. Riesner and Römer[1] discuss mainly theoretical aspects of the complex formation and the theory of the formation of "hairpin" structures. Ts'o's review[2] (1974) is devoted mainly to the consideration of complexes with non-canonical pairs of bases—the self-associated species oligo(A) [U, C, or G]. The review of Cantor and Schimmel[3] deals with the statistical thermodynamics and kinetics of the formation of duplexes by oligoribonucleotides (including mainly the hairpin and loop structures) and the employment of such data for the prediction of the secondary structure RNA as well as the kinetics of the denaturation and renaturation of DNA. The results of the crystallographic study of short oligomeric duplexes are discussed in Seeman's review[4]. In addition, the complexes of oligonucleotides are mentioned in reviews and other topics: on the interaction of mono(oligo)nucleotides with polynucleotides[5]; on the structure of RNA[6]; on the study of polynucleotides[7] and single-stranded oligonucleotides by NMR[8]. The present review embraces virtually all the aspects of the complex formation by oligonucleotides containing from 2 to 15 monomeric units (including studies up to 1981). Particular attention has been devoted to the results obtained in recent years in the study of the interaction of oligonucleotides by NMR and data on the structure of the oligomeric duplexes. The review does not discuss in detail studies dealing with the formation of "hairpin" structures and complexes of oligonucleotides containing non-complementary bases.

II. TYPES OF OLIGOMERIC COMPLEXES INVESTIGATED

The oligomeric complexes can be formed by oligonucleotides of identical or different lengths with complementary sequences or by self-complementary oligonucleotides. Depending on the nucleotide sequence and the chain length in these systems, the formation of several types of complexes has been noted.

Complexes of the readily available synthetic oligoribonucleotides with a homogeneous composition have been investigated most completely: oligo(A).oligo(U)[9–11] [the complexes oligo(G).oligo(C) have not been investigated hitherto]. Oligomers with a homogeneous sequence tend to form triple-stranded helices capable of coalescing into aggregates of the "concatameric" type with a higher molecular weight (see below)[9,11]. When the short oligomers of adenylic and uridylic acids interact, the stoichiometry of the complexes remains 1A:2U regardless of the chosen conditions[9]. It has been demonstrated[11] that the chain length of the oligomers, i.e. the number of monomeric units involved in the complementation interactions (N) above which there is a possibility of the formation of double helices, is seven. Systems incorporating oligodeoxyribonucleotides form as a rule double-stranded helices[12]. Thus the hybrid oligomeric complex $d(pT)_8.r[A(pA)_7]$ has the stoichiometry 1T:1A in a neutral buffer containing 1 M NaCl.[13] A double helix structure is also characteristic of a complex of oligodeoxyribonucleotides of different lengths: $d(pT)_{12}.d(pA)_6$.[14]

Oligomers with an alternating self-complementary nucleotide sequence usually form either intermolecular double helical complexes, such as, for example, oligo [d(pGG–C)] with a chain length ranging from 4 to 7 monomeric units[15], or intramolecular complexes as a result of the folding of the oligomer chain: $d(A–T)_n$ ($n > 7$).[16] The particular structure of the complex is apparently determined by the length of the oligomer. In the formation of complexes by oligonucleotides of different lengths with alternating non-complementary bases, namely oligo[d(C–A)].oligo[d(T–G)],[17] as well as trinucleotides and oligomers containing repeating trinucleotide sequences, namely $d(pG–G–T).d(pA–C–C)_{2-4}$, etc.,[18] the formation of double helix structures with continuous filling of the matrix by shorter oligomers is favoured.

Detailed study of the structural characteristics of the complexes of self-complementary block copolymers $(Ap)_mApU(pU)_n$[19] revealed a dependence of the structure of the complexes on the A/U ratio in the oligomer chain. A double-stranded helix is formed for $m \geq n$, while larger aggregates containing triple-stranded sections are produced for $m < n$.

The interaction of oligonucleotides with a heterogeneous nucleotide sequence leads to the formation of short double helix duplexes with a fixed position of one nucleotide chain relative to the other[21–26], provided that such interaction is not complicated by the self-aggregation of the components, as in the case of the complex G–G–C.G–C–C.[20] It has been observed recently that the hybrid ribo-deoxyriboduplex $r(C–U_5–G).d(C–A_5–C)$ has a triple helix (2:1) structure[27].

If the primary structures of the heterogeneous oligomers permit the formation of duplexes with "sticky" ends, for example, d(G–A–T–C–C–G–A–A–T–T–C–G)[28], d(T–G–C–A–C–A–T–G)[29], d(T–G–G–C–C–A–A–G–C–Tp)[30,31], and $d(A–T)_3(C–G)_3$,[32] etc.,[33] then the self-association of such oligomers results in the formation of double helices of the "concatameric" type. Concatamers are double helix complexes containing repeating nucleotide sequences. The structure of the concatamers formed by the above oligonucleotides resembles that of the polymeric complexes but differs from it by the presence of non-coincident breaks in the complementary chains.

III. THERMODYNAMIC DESCRIPTION OF THE COMPLEX FORMATION BY COMPLEMENTARY OLIGONUCLEOTIDES

1. General Principles of the Cooperative Interaction of the Oligomers

According to modern ideas, the characteristics of the behaviour of double (triple) helix nucleotide structures in solution are determined by the interaction between the heterocyclic bases, the influence of the solvent, and the electrostatic effects of the phosphate groups. The forces involved in the complex formation have been considered theoretically in a monograph[34]. The change in the state of each covalently bound monomeric unit in the complementary complex does not take place independently of the neighbouring units, which is the reason for the cooperative character of the formation and dissociation of the double (triple) helix systems. The compact packing of the heterocyclic bases of the double helix is drastically impaired in the dissociation of the complex, which is reflected in the cooperative alteration of many physicochemical characteristics of the system[5]—the absorption ultraviolet and infrared spectra, the circular dichroism (CD) and optical rotatory dispersion (ORD) parameters, NMR signals, and also hydrodynamic parameters, etc. The curves for the temperature variation of these parameters are referred to as the melting curves and make it possible to estimate the fraction of paired bases (f) at a given temperature. For example[19]:

$$f(T) = \frac{A_s(T) - A(T)}{A_s(T) - A_d(T)},$$

where $A_S(T)$ and $A_d(T)$ are the ultraviolet absorbances of the denatured and native complexes respectively at the given temperature, $A(T)$ is the ultraviolet absorbance of the solution observed at the given temperature (a point on the melting curve—Fig.1). T_m is defined as the temperature at which $f = 1/2$.

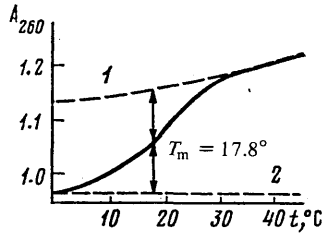
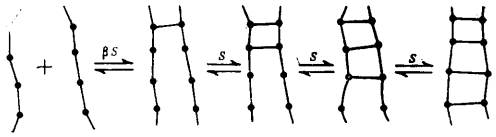


Figure 1. Temperature variation of the optical density of the intermolecular complex formed by $(Ap)_4ApU(pU)_4$ (1 M NaCl, pH 7.4): 1) $A_S(T)$; 2) $A_d(T)$.[19]

The statistical theory of the helix–coil transition, based on a modified monomeric Ising model[35], has now been fairly thoroughly developed[36] and is used to obtain the thermodynamic parameters of the complex formation process from the analysis of the experimental melting curves. Within the framework of this theory, the cooperative properties of the complementary oligomeric complexes, incorporating a single type of base pairs, can be described by a simple model with three parameters: the nucleation parameter β of the double helix ($\beta \ll 1$); the equilibrium constant S of the formation of the nucleotide pair next to a double helix sequence already

formed ($S > 1$); the number N of base pairs in the duplex. According to Applequist and Damle[37], the formation of the helix can be represented schematically as follows for the tetramer:



where βS is the formation constant of the isolated base pair (it determines the helix initiation process). The essential feature of the theory consists in the fact that the base pairs within the structure are much more stable than the terminal pairs and the initiation of the double helix therefore leads to the rapid complementary pairing of the bases of the entire nucleotide chain. However, the free energy gain for fairly long nucleotide sequences, which determines the increase in the length of the double helix, is compensated by the favourable entropy characteristics of the incompletely bound structures. The statistical weight of such structures increases sharply with increasing chain length. Indeed, whereas the formation of a complete double helical complex or a fully dissociated coil can occur in only one way, one can imagine a multiplicity of intermediate structures containing half of the theoretically possible nucleotide pairs. The competition between the above two factors leads to a finite average length L of the helical segments, in the region of the helix–coil transition, alternating with the melted sections; $L \cong 20$–100 nucleotide residues[1]. In the case of fairly short oligomeric sequences, with $N \ll L$, the complex formation process can be described with the aid of the "all or nothing" model (the two-state model), neglecting the number of oligonucleotides in which not all the bases are linked within the complex.

2. The Two State Model

Complex formation in accordance with the "all or nothing" principle takes place for fairly large helix propagation constants $S > 10$ (Pörschke[11]) ($\beta \ll 1$). Within the framework of this model, it is assumed that in the range of the helix–coil transition short or even nucleotides exist in only two states—in the fully double helix state or in the free state. The equilibrium constant K for the complex formation by the oligonucleotides containing N monomeric units can be formulated as the product of the constants for the individual stages of the reaction[15]:

$$K = \beta S^N \ .$$

The equilibrium constant can also be expressed in terms of f (Borer et al.[23]):

$K = f/2(1-f)^2c$ (interaction of self-complementary chains);

$K = 2f/(1-f)^2c$ (interaction of non-identical complementary sequences of the same length),

where c is the overall nucleotide concentration of the oligomeric components.

The equilibrium constant of the interaction of trinucleotides with complementary oligomeric matrices, containing repeating trinucleotide sequences[18], as well as the equilibrium constant of the formation reaction of concatamers with symmetrically overlapping blocks[29] were derived recently. The fraction of paired bases can be determined directly with the aid of osmometric experiments. A more convenient method of determination of f consists in the analysis of fusion curves.

The thermodynamic description of the complex formation process usually involves the calculation of $\Delta H°$, $\Delta S°$, β, and S.

Within the framework of the two-state model, the enthalpy of reaction can be determined as follows.

1. From the temperature variation of the equilibrium constant (by the van't Hoff method):

$$\ln K = -\Delta H°/RT + \Delta S°/R \ .$$

2. From the concentration variation of T_m[15,19]:

$$1/T'_{пл} = 1/T''_{пл} + R/\Delta H° \cdot \ln c'/c'' \ .$$

We may note that this method is applicable only to the description of intermolecular interaction, because intramolecular complex formation is independent of concentration.

3. From the slope of the melting curve at the point corresponding to T_m (Podder[21]):

$$(\partial f/\partial T)_m = \Delta H°/6RT_m^2 \ .$$

If the melting curve is expressed in a differential form, the formula relating the enthalpy of reaction to the half-width of the transition (T_1–T_2) can be written as follows[38]:

$$\Delta H° = -5.106 R T_1 T_2/(T_2 - T_1),$$

where T_1 and T_2 are the temperatures (K) at which $(\partial f/\partial T^{-1}) = 0.5(\partial f/\partial T^{-1})_{max}$. The enthalpy of complex formation determined by the second method agrees well with $\Delta H°$ obtained by direct calorimetric measurements [this has been demonstrated for the duplexes $d(G-C)_3$[39] (Table 1, system 9) and A_7U_7[40]].

The determination of the heat of formation of oligomeric duplexes with different numbers of base pairs makes it possible to estimate the enthalpy change per nucleotide pair[15]:

$$\Delta H° = \Delta H°_{\beta S} + (N-1)\Delta H°_S \ ,$$

where the indices βS and S denote respectively the thermodynamic parameters of the formation of the first and subsequent nucleotide pairs. If it is assumed that the stability of the oligomeric duplex is proportional to the number of monomeric units, then $\Delta H° = N\Delta H°_1$ and $\Delta S° = N\Delta S°_1$, where $\Delta H°_1$ and $\Delta S°_1$ are the thermodynamic parameters of the formation of the nucleotide pair on the assumption that all the base pairs are identical[19].

Since T_m for the duplex is usually measured more accurately than $\Delta H°$ and $\Delta S°$, it is useful to examine the dependence of T_m on the length of the oligomeric duplex. The direct proportionality between $1/T_m$ and $1/N$ has been predicted[37]:

$$1/T_m = 1/T_m^\infty + R \cdot \ln(\beta c)/N\Delta H°_1 \ ,$$

where T_m^∞ is the melting point of the corresponding polymeric complex and c the concentration of the oligomeric components. The slope of the $1/T_m - 1/N$ straight line and the intercept on the ordinate axis make it possible to determine the nucleation parameter and T_m respectively subject to the condition that $\Delta H°_1$ is known. Here it is assumed that $\Delta H°_1$ and β are constant. In real nucleotide systems the dependence of $1/T_m$ on $1/N$ frequently deviates from linearity[13,15], which indicates a temperature variation of β. If this is true, the enthalpies of the initiation ($\Delta H°_{\beta S}$) and propagation ($\Delta H°_S$) of the helix should differ. The temperature dependence of β is taken into account by introducing the stacking factor χ, which determines the relative contribution of the interplanar interactions to the enthalpy of propagation of the double helix[41]: $\Delta H°_\beta = -\chi\Delta H°_S$. The modified equation of Applequist and Damle has the following form[1]:

$$1/T_m = 1/T_m^\infty + 1/N - \chi R \ln(\beta S^\chi c)/\Delta H°_\beta \ .$$

The equation includes two limiting cases: the enthalpy of initiation is equal to the enthalpy of propagation of the helix ($\chi = 0$) and the enthalpy of initiation is zero ($\chi = 1$). The

best agreement with experimental data for the complexes oligo(A).oligo(U) is obtained when $\chi = 0.7$,[11] for $d(pG-C)_n$[15] and the hybrid complexes $d(pT)_8.rA(pA)_7$[13] when $\chi = 1$, and for the oligo(A) double helices in an acid medium when $\chi = 0$.[41]

The two-state model constitutes a good approximation for the calculation of the thermodynamic parameters of the complex formation by short oligomers. However, in each specific case it is useful to estimate the validity of the applicability of this model. The conditions governing the validity of the two-state model have been described in a review[1].

3. The Model Taking into Account the Set of States (the "Staggered Zipper Model")

In the calculation of the thermodynamic parameters of the complex formation by fairly long oligomers ($N \cong L$), it is no longer permissible to neglect the concentration of the oligonucleotides where not all the bases are linked in the complex. In order to describe the thermodynamics of such systems, Applequist and Damle[37] proposed a model taking into account the set of partially bound structures. Taking into account the equilibrium constants for each state βS^z (z is the number of paired bases), it is possible to derive the overall equilibrium constant of the complex formation reaction:

$$K_z = \beta\chi(z, N)S^z \ ,$$

where $\chi(z,N)$ is the number of possible states of the complex with z nucleotide pairs. The proposed model makes it possible to estimate quantitatively the populations of different states (for further details of the theory, see Applequist and Damle[37]). The formation of the oligo(A) double helix in an acid medium[41] as well as the complex formation by $d(A-T)_n$[16], oligo(A) + oligo(U)[11], and a large set of heterogeneous oligoribonucleotides[22] have been described thermodynamically with the aid of this model (Table 1). For comparatively short oligomeric complexes (up to 10 monomeric units), the experimental fusion curves are described satisfactorily, as stated above, by the simpler two state model and the thermodynamic parameters of the reaction obtained with the aid of the two models are very similar[22]. Since the direct application of the "staggered zipper model" is fairly complex in the interpretation of experimental data, the "all or nothing" model is usually employed as a first approximation in the determination of thermodynamic parameters. Subsequently the results are corrected on the basis of the analysis of the populations of the intermediate states.

4. The Introduction of Corrections in the Calculation of Thermodynamic Parameters

Evidently in the temperature range corresponding to the melting of oligomeric duplexes (usually at comparatively low temperatures) there is equilibrium between the double helix complex and the single stranded components in which the interplanar interactions are only partly affected. The intrinsic secondary structure of the oligomeric strands is determined by the stacking interactions of the bases, which weaken with increase of temperature[42,43]. Thus the non-cooperative process involving the breakdown of the stacking conformations of the single-stranded components is superimposed on the helix–coil transition in the oligomeric systems. In order to compare the thermodynamic parameters of the complex formation reactions in different systems, it is necessary to eliminate the stacking interactions of the oligomers, i.e. to begin the reckoning from the fully destructured state.

The contribution to the free energy of the helix–coil transition due to the breakdown of the stacking conformations of the single-stranded oligonucleotides can be represented as follows[11,22]:

$$\Delta G^0_{\text{stacking}} = RT \ln (1 + e^{\Delta G^0_A/RT})(1 + e^{\Delta G^0_B/RT}) \ ,$$

where ΔG°_A and ΔG°_B are the free energies of the stacking interaction between neighbouring bases in the complementary oligomeric chains (A and B). It is fairly difficult to calculate ΔG°_A and ΔG°_B. For this purpose, it is necessary to examine the transition of each interacting oligomer from the ordered to the disordered state and to calculate the enthalpy and entropy of this process. A simpler approach involves the use of the thermodynamic parameters of the stacking interaction of dinucleotide phosphates[13,22] This type of correction of thermodynamic data has been made for a series of oligoribonucleotide systems[11,13,22] (Table 1). It follows from the tabulated data that the enthalpies and entropies of complex formation of oligoribonucleotides calculated with and without allowance for the breakdown of the stacking conformations of the single-stranded components differ appreciably. This shows that at the melting point of the complex the intrinsic secondary structure of the oligoribonucleotide chains is only slightly affected. Since the thermal stability of the stacking conformations in oligodeoxyribonucleotides is much lower than in oligoribonucleotides[44,45], the corresponding energy contribution is not significant in the calculation of the thermodynamic parameters of the complex formation by the DNA oligomers[15]. It is of interest to note that the fraction of the stacking conformations of the single-stranded components diminishes after the addition of up to 10% of alcohols, urea, etc. to aqueous solutions[46].

The statistical theory developed by Warshaw and Cantor[47] predicts that the stacking conformations of the single-stranded oligomers are the most important of the three factors which determine the difference in the behaviour of short double helices and polymer systems. The two other factors are the influence of the terminal nucleotide pairs and the effects of the neighbouring base pairs, which are characteristic of heterogeneous oligomeric duplexes.

Electrostatic interactions also influence the free energy of complex formation. Theoretical examination of the interaction of the negative charge of the phosphate groups of the complementary nucleotide chains with the counterions predicts a linear increase of T_m with increase of the logarithm of the concentration of univalent ions in polymer systems[38,48]. For oligomeric complexes, some deviation is observed from the linear relation [for example, in the oligo(rA).oligo(dT) system[13]]. The influence of the length of the chain of the oligomeric complex on the relation between T_m and $\lg I$ (I is the ionic strength of the solution) has also been noted[14,15].

The contribution of electrostatic interactions to the free energy of the helix–coil transition is usually described by the equation[11,13,48]

$$\Delta G^0_{\text{electr}} = RT(C_1 - C_2 \lg I) \ ,$$

where C_1 and C_2 are empirical constants. According to the data of Hogget and Maass[13], the constant C_2 for oligomeric complexes can be estimated from the dependence of the stability constant of the double helix on the ionic strength of the solution.

Thus the change in free energy as a result of the increase in the length of the double helix by one nucleotide pair (ΔG°_S) can be expressed as follows taking into account the stacking interactions in the single-stranded oligomers and the electrostatic interactions[11,13]:

$$\Delta G^0_S = \Delta G^0_{12} + \Delta G^0_{\text{stacking}} + \Delta G^0_{\text{electr}} \ ,$$

Table 1. Thermodynamic parameters of the complex formation by complementary oligonucleotides.

No.	Oligomeric system	Complex formation conditions	Melting temperature range or T_m, °C	β, litre mol^{-1}	S	$\Delta H°$, kcal mol^{-1}	$\Delta S°$, e.u.	$\Delta H°_S$, kcal mol^{-1}	$\Delta S°_S$, e.u.	Refs.
1	A(pA)$_n$+U(pU)$_n$, N=7—17	0.5 M cacodylate buffer, pH 6.9	10—35	$5\cdot10^{-4}$—10^{-3}	7.9(0°)	—	—	—10.9	—30	[11]
2	d(pT)$_7$+rA(pA)$_6$	0.1 M cacodylate buffer, pH 7.0	5—30	10^{-4}—$5\cdot10^{-4}$	21(0°)	—73.5	—218	—11.2	—30.7	[13]
	d(pT)$_8$+rA(pA)$_7$	1 M NaCl	5—30	10^{-4}—$5\cdot10^{-4}$	21(0°)	—84	—253	—11.2	—30.7	
	d(pT)$_9$+rA(pA)$_8$		5—30	10^{-4}—$5\cdot10^{-4}$	21(0°)	—90	—264	—11.2	—30.7	
3	GpGpGpC+GpCpCpC	0.1 M phosphate buffer, pH 7.2	20—40	10^{-3}	~100 (22—39°)	—21.6	—50	—6—7	—12.5	[21]
4	(Ap)$_3$ApU(pU)$_3$	0.01 M phosphate buffer, pH 7.0, 1 M NaCl, 10^{-4} M EDTA; $c = 10^{-4}$ M	12	$1.4\cdot10^{-4}$	—	—54(—46)	—	—6.4(—5.8)	—19.8	[19]
	(Ap)$_4$ApU(pU)$_4$		23	$1.4\cdot10^{-4}$	—	—72(—53)	—	—7.2(—5.3)	—19.8	
	(Ap)$_5$ApU(pU)$_5$		32	$1.4\cdot10^{-4}$	—	—85(—56)	—	—7.1(—4.8)	—19.8	
	(Ap)$_6$ApU(pU)$_6$		40	$1.4\cdot10^{-4}$	—	—107(—72)	—	—7.6(—5.1)	—19.8	
5	(Ap)$_3$CpG(pU)$_3$	0.01 M phosphate buffer, pH 7.0, 1 M NaCl, 10^{-4} M EDTA; $c = 10^{-4}$ M	35.3	$2.2\cdot10^{-4}$	—	—65	—	—7.6	—	[49]
	(Ap)$_4$CpG(pU)$_4$		42.5	$2.2\cdot10^{-4}$	—	—76	—	—7.6	—	
	N=6									
	A$_4$CG+CGU$_4$	0.01 M phosphate buffer, pH 7.0, 1 M NaCl, 10^{-4} M EDTA; $c = 10^{-4}$ M ($A+A$ helix) and 2×10^{-4} ($A+B$ helix)	~1.3	—	—	—26	—75.8	see Table 2	see Table 2	[23]
	U$_2$CA$_2$		11.3	—	—	—37	—112			
	A$_2$CU$_2$		22.1	—	—	—34	—96.9			
	A$_4$G$_2$+C$_2$U$_4$		22.8	—	—	—44	—129			
	A$_2$CGU$_2$		28.3	—	—	—46	—134			
	N=7									
6	A$_3$CU$_3$+A$_3$GU$_5$	ditto	16.4	—	—	—41	—122	ditto	ditto	[23]
	N=8									
	A$_4$U$_4$	»	11.5	—	—	—51	—160	»	»	[23]
	A$_4$CU$_3$+A$_3$GU$_4$		22.1	—	—	—52	—156	»	»	
	A$_3$CGU$_3$		35.1	—	—	—66	—196			
	A$_3$GCU$_3$		42.3	—	—	—53	—150			
	N=9									
	A$_4$CU$_4$+A$_4$GU$_4$	»	26.0	—	—	—58	—174	»	»	[23]
	N=10									
	A$_5$U$_5$	»	24.3	—	—	—67	—207	»	»	[23]
	A$_4$UAU$_4$		27.7	—	—	—72	—221			
	A$_5$CU$_4$+A$_4$GU$_5$	»	32.6	—	—	—63	—187			
	A$_4$CGU$_4$		41.9	—	—	—76	—223			
	A$_4$GCU$_4$		46.8	—	—	—78	—225			
	N=11									
	A$_5$CU$_5$+A$_5$GU$_5$	»	35.4	—	—	—89	—269	»	»	[23]
	N=12									
	A$_6$U$_6$	»	31.5	—	—	—84	—257	»	»	[23]
	N=14									
	A$_7$U$_7$	»	39.3	—	—	—107	—324	»	»	[23]
7**	A$_2$GCU$_2$	0.05 M cacodylate buffer, pH 7.0; $c = 10^{-5}$ M ($A+A$) and 2×10^{-5} M ($A+B$)	8	$5\cdot10^{-4}$	170	—72	—215	(—14)—15	—38.0	[22]
	A$_3$GCU$_3$		20	$5\cdot10^{-4}$	200 } 170	—92	—268	(—12)—15	—36.8	
	A$_4$GCU$_4$		25	$5\cdot10^{-4}$	140	—111	—326	(—12)—15	—37.7	
	A$_6$CG+CGU$_6$		7	10^{-3}	67 } 60	—95	—289	(—15)—15	—41.2	
	A$_8$CG+CGU$_8$		16	10^{-3}	53	—102	—304	(— 9)—14	—38.0	
	A$_4$G$_2$+C$_2$U$_4$		1	10^{-3}	110 } 130	—76	—233	(—16)—15	—39.5	
	A$_5$G$_2$+C$_2$U$_5$		6	10^{-3}	150	—87	—265	(—16)—17	—47.1	
	A$_4$G$_3$+C$_3$U$_4$		22	10^{-3}	108 } 95	—76	—216	(—11)—11	—25.2	
	A$_5$G$_3$+C$_3$U$_5$		24	10^{-3}	86	—88	—254	(—11)—11	—27.3	
8*	GpApGpC+GpCpUpC	0.01 M phosphate buffer, pH 7.0, 0.1 M NaCl, 10^{-4} M EDTA; $c = 10^{-4}$ M ($A+A$) and 2×10^{-4} M ($A+B$)	17.7	—	—	—24.4(—39.1)	—	—	—	[50]
	GpApGpC+ApGpCpUpC		16.0	—	—	—24.4(—30.7)	—	—	—	
	ApGpCpU pC		6.0	—	—	—29.3(—25.9)	—	—	—	
9	oligo d(pG—C) N=4—7	0.045 M phosphate buffer, pH 7.2, 1 M NaCl, 2×10^{-3} EDTA	10—70	$1.6\cdot10^{-4}$	104±5 (25°)	—28.8 (N=4) —38.5 (N=5) —50.7 (N=6) —60.2 (N=7)	—	—11.1±0.5	—27.9±1	[15]
	d(G—C)$_3$*	0.045 M cacodylate buffer, pH 7.0, 1 M NaCl; $c = 2.9\times10^{-5}$ M	50	—	—	—56.9(—59.6)	—	—	—	[39]
	d(G—C)$_3$*	0.045 M cacodylate buffer, pD 7.3, 1 M NaCl (D_2O)	—	—	—	—62.7(—65.8)	—	—	—	[39]
10	d(A—T—G—C—A—T)	cacodylate buffer, pD 7.0 (D_2O)	20—40	—	—	—34	—	—	—	[51]
11	d(T—T—G—T—T)+ +d(A—A—C—A—A)	0.1 M phosphate buffer, pD 7.0, 0.15 M NaCl (D_2O)	0—30	—	—	—34	—	—	—	[24]
12	d(G—G—A—A—T—T—C—C)	0.01 M phosphate buffer, pH 6.85, 10^{-3} M EDTA, 0.1 M NaCl; $c = 5.3\times10^{-3}$ M	46±3	—	—	—51.8	—151	—	—	[52]
13	dC(pA)$_5$pG+dC(pT)$_5$pG	0.01 M phosphate buffer, pH 7, 10^{-4} M EDTA, 0.2 M NaCl; $c = 2\times10^{-4}$ M	27.5	—	—	—41±4	—	—	—	[53]
	rC(pA)$_5$pG+dC(pT)$_5$pG		22.9	—	—	—48.4	—	—	—	
	rC(pA)$_5$pG+rC(pU)$_5$pG		19.1	—	—	—44±4	—	—	—	
	dC(pA)$_5$pG+rC(pU)$_5$pG		<0	—	—	—	—	—	—	
14***	d(pA—C—C)$_2$+ +d(pG—G—T)	0.004 M phosphate buffer, pH 7.3, 0.2 M NaCl, 0.075 M $MgCl_2$; $c = 0.8\times10^{-2}$ M	11(9)	$0.5\cdot10^{-3}$	35(10°)	—65 (—54)	—197 (—163)	—10.4	—30.6	[18]
	d(pA—C—C)$_3$+ +d(pG—G—T)		18(18)	$0.5\cdot10^{-3}$	20(16°)	—98 (—87)	—288 (—262)	—10.4	—30.6	
	d(pA—C—C)$_4$+ +d(pG—G—T)		22(23)	$0.5\cdot10^{-3}$	19(23°)	—122(—119)	—355 (—343)	—10.4	—30.6	
	d(pA—C—C)$_2$+ +d(pT—G—G)		9(9)	$0.5\cdot10^{-3}$	—	—(—47)	—(—138)	—10.4	—30.6	
	d(pA—C—C)$_3$+ +d(pT—G—G)		18(18)	$0.5\cdot10^{-3}$	32(16°)	—(—78)	—(—226)	—10.4	—30.6	
	d(pA—C—C)$_4$+ +d(pT—G—G)		21(22)	$0.5\cdot10^{-3}$	23(22°)	—(—106)	—(302)	—10.4	—30.6	
	d(pC—C—A)$_2$+ +d(pG—G—T)		9(9)	$0.5\cdot10^{-3}$	59(9°)	—64 (—58)	—198 (—176)	—10.4	—30.6	

Table 1 (Cont'd.)

No.	Oligomeric system	Complex formation conditions	Melting temperature range or T_m, °C	β, litre mol^{-1}	S	$\Delta H°$, kcal mol^{-1}	$\Delta S°$, e.u.	$\Delta H°_S$, kcal mol^{-1}	$\Delta S°_S$, e.u.	Refs.
	d(pG—C—A)$_2$+ +d(pT—G—G)		8(9)	$0.5\cdot10^{-3}$	—	—(—60)	—(—183)	—10.4	—30.6	
	d(pT—G—G)$_2$+ +d(pC—C—A)		3(2)	$0.5\cdot10^{-3}$	—	—(—65)	—(—204)	—10.4	—30.6	
	d(pA—C—C)$_2$+ +d(pT—G—G)$_2$		13(15)	$0.5\cdot10^{-3}$	—	—(—53)	—(—169)	—10.4	—30.6	
15***	d(T—G—C—A—C—A—T—G)	0.004 M phosphate buffer, pH 7.3, 0.2 M NaCl, 0.075 M $MgCl_2$; $c = 10^{-3}$ M	20	—	—	—74.0±2.4	—216±8	—9.2±0.3	—27±1	[29]

Note. The thermodynamic parameters of systems 1, 2, and 8 were calculated taking into account the breakdown of the stacking conformation of the single-stranded components. The two-state model (systems 2–6 and 8–15), the model taking into account the set of states (system 1), or both models (system 7) were used in the calculation (the values of $\Delta H°_S$ calculated on the basis of the two-state model are given in brackets); the abbreviations for the oligonucleotides were taken from the original communications.

* The values of $\Delta H°$ and $\Delta H°_S$ calculated in different ways are quoted.

** The values of $S°$, $\Delta H°_S$, and $\Delta S°_S$ refer to the formation of the G:C pairs.

*** The values of T_m, $\Delta H°$, and $\Delta S°$ calculated from the temperature variation of the CD parameters are quoted; the values evaluated from the temperature variation of the ultraviolet absorption are given in brackets.

**** The values of $\Delta H°$ and $\Delta S°$ for $n = 3$ (n is the number of octanucleotide molecules involved in the formation of the concatamer) are quoted.

where $\Delta G°_{12}$ is the energy of formation of the additional nucleotide pair from the statistical coil with uncharged phosphate groups.

The chain propagation constant $S = e^{\Delta G°_S/RT}$. The breakdown of the stacking conformations of the single-stranded oligomers and electrostatic interactions also influence the initiation of the double helix[11].

The thermodynamic parameters of the formation of oligomeric complexes of different composition and length, calculated resorting to different models, are summarised in Table 1. The first detailed thermodynamic studies of the complex formation by oligoribonucleotides were undertaken by Pörschke[11] [oligo(A).oligo(U)] and in the studies of Martin et al.[19] and Uhlenbeck et al.[49] [$(Ap)_nApU(pU)_n$ and $(Ap)_nCpG(pU)_n$]. Subsequently oligomeric duplexes with a greater variety of compositions and nucleotide sequences were investigated. The thermodynamic parameters of 19 RNA duplexes containing between 6 and 14 nucleotide pairs have been reported[23]. The thermodynamics of the formation of DNA duplexes have also been investigated: self-complementary oligomers with the repeating dinucleotide sequences $d(A-T)_n$[16] and $d(pG-C)_n$[15], with the heterogeneous sequences d(A—T—G—C—A—T)[51], d(G—G—A—A—T—T—C—C)[52], etc. (Table 1). A thermodynamic description of the formation of double helical oligodeoxyribonucleotide complexes with breaks in the chain of phosphodiester bonds, including complexes of the concatameric type, has been achieved recently[18,29,30].

IV. CHARACTERISTIC FEATURES OF THE THERMODYNAMICS OF COMPLEX FORMATION BY HETEROGENEOUS OLIGONUCLEOTIDES

1. The Stability of the A:U(T) and G:C Pairs

The increase of the thermal stability of natural DNA and synthetic double helix polynucleotides with increase of the relative content of the G:C pairs is a well known experimental fact[54]. The correlation between the content of the G:C pairs and the stability of the helices has been demonstrated also at the level of oligomeric complexes. Thus, in a study[49] on the stability of the complexes of the block copolymers $(Ap)_nApU(pU)_n$, $(Ap)_nCpG(pU)_n$, and $(Ap)_nG(pU_n)(Ap)_n\cdot C(pU)_n$, it was shown that T_m of the double helices increases in direct proportion to the percentage content of the G:C pairs; with increase of the content of such pairs by 1%, T_m of the complexes increases on average by 0.9°. For natural DNA and RNA, the corresponding increments are 0.5° and 0.75° respectively. The enhanced stability of the G:C pairs in the oligomeric duplexes is believed[49] to be caused by the fact that the nucleotide sequence of the base pairs in the duplexes is far from the quasi-statistical distribution characteristic of the natural nucleic acids. The enthalpy of complex formation by $(Ap)_nCpG(pU)_n$ is significantly higher than the enthalpy of complex formation by $(Ap)_nApU(pU)_n$: −65 and −54 kcal mol^{-1} respectively (for $N = 8$). According to the authors' estimate[49], $\Delta H°_{G:C}$ is higher by ~ 3–4 kcal mol^{-1} than $\Delta H°_{A:U}$.

The study of duplexes containing complementary pairs of one type permitted the calculation of the thermodynamic parameters of the formation of the A:U(T) or G:C pairs. The value of $\Delta H°_{G:C}$ obtained from the study of the complex formation by $d(pG:C)_n$[15], which is −11.1 kcal mol^{-1}, greatly exceeds $\Delta H°_{A:U}$, which has been estimated as −8 kcal mol^{-1},[16] while the free energy of a single G:C pair at 37 °C corresponds to the free energy of two A:T pairs. Fig.2 presents the dependence of the thermodynamic parameters of the formation of nucleotide pairs on the composition of the deoxyribonucleotide duplexes.

The enthalpies of formation of the G:C and A:U pairs obtained with and without allowance for the stacking interactions in the oligomers have been compared[22]. In a study of the complex formation by a large set of oligoribonucleotide with a heterogeneous composition, it was established[22] that the enthalpy of formation of the G:C pair from a fully destructured coil (−15 kcal mol^{-1}) is higher than the corresponding value for the A:U pair (−10.7 kcal mol^{-1}). If a correction taking into account the temperature dependence of the stacking conformation of the single-stranded oligomers

is applied in the calculation of the enthalpy, then the difference between $\Delta H^{\circ}_{G:C}$ (−5 − −6 kcal mol^{-1}) and $\Delta H^{\circ}_{A:U}$ (−5 − −6 kcal mol^{-1}) virtually disappears, because the two bases in the G:C pairs interact with the neighbouring bases, whereas the stacking interactions in the A:U pair with participation of uracil are insignificant.

The stability constants S of the G:C and A:U(T) pairs have been calculated for a series of ribo- and deoxy-ribonucleotide complexes (Table 1). Comparison of $S_{G:C}$ (100–200) with $S_{A:U}$ (~ 10) shows that the G:C pair is on average 15 times more stable than the A:U(T) pair.

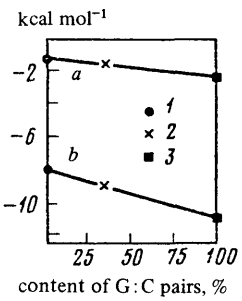


Figure 2. ΔG°_{S} (*a*) and ΔH°_{S} (*b*) for the formation of nucleotide pairs in DNA of different composition at 37 °C (1 M NaCl, pH 7.0): 1) poly d(A–T); 2) T-2:phage DNA; 3) oligo [d(pG–C)].[15]

2. The Influence of the Nucleotide Sequence and Other Factors on the Stability of the Duplexes

It follows from the analysis of the data in Table 1 (system 7) that $S_{G:C}$ depends on the nucleotide sequence of the oligomeric components, i.e. the stability of the oligomeric complexes is influenced not only by the length of the helix and the nucleotide composition but also by the sequence of the base pairs in the chain (nucleotide environment). The dependence of the thermal stability of nucleotide complexes on the base pair sequence was observed for the first time for synthetic polymer systems with a repeating di(tri)nucleotide sequence[55,56].

According to modern ideas[23], the difference between the stabilities of the isomeric complexes is due to the different geometries of the "stacking" of neighbouring base pairs in the helical structures. Theoretical calculations predict that the contribution of each base pair to the stability of the complex depends mainly on the nearest neighbouring base pairs, although in real systems it is frequently impossible to neglect the contribution of long-range interactions. Evidently the influence of the nucleotide sequence should be manifested most strikingly in the properties of short oligomeric complexes. Indeed, when five isomeric helices containing two G:C and four A:U pairs are compared, the T_m of these complexes under the conditions of identical nucleotide concentrations vary from −1.3° to 28.3 °C.[23] An increase in the length of the nucleotide chain leads to a gradual averaging of the thermodynamic parameters and in the limit this effect is not manifested for polymeric structures with a statistical distribution of bases along the chain.

By considering only the Watson–Crick A:U(T) and G:C interactions, it is possible to formulate 10 different combinations of two nucleotide pairs, i.e. 10 different ways in which the interplanar interactions between two base pairs in the double helix can take place:

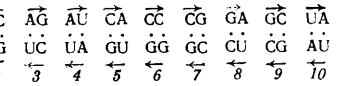
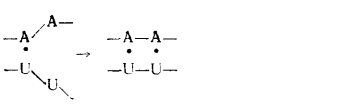
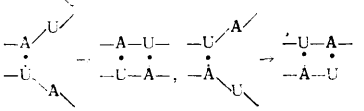
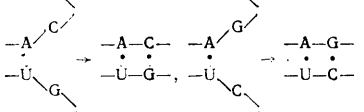
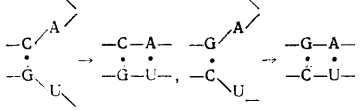
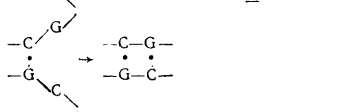
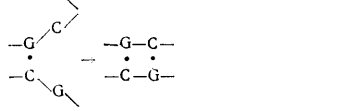


Table 2. Thermodynamic parameters of the formation of the nucleotide pair next to a base pair already formed (for all possible combinations of two Watson–Crick pairs)*[23].

Reaction	ΔH^{0}_{S}, kcal mol^{-1}	ΔS^{0}_{S}, e.u.	ΔG^{0}_{S}, kcal mol^{-1} 25 °C
	−8.2	−23.5	−1.2
	−6.5	−16.4	−1.6
	−5.9	−12.7	−2.1
	−13.0	−33.5	−3.0
	−14.7	−34.9	−4.3
	−13.7	−29.8	−4.8

* The calculation was based on the thermodynamic data presented in Table 1 for these systems[23].

Gralla and Crothers[57] isolated three combinations of two pairs of bases—two neighbouring A:U pairs in any order, two neighbouring G:C pairs in any order, and two neighbouring A:U and G:C pairs in any order, and obtained three equilibrium constants for the description of the helix–coil equilibrium on the basis of the interaction of the neighbouring pairs. The study of the thermodynamics of the series of oligomeric duplexes of different compositions and with different nucleotide sequences permitted[23] the calculation of the thermodynamic contributions of six combinations of nucleotide

pairs. Table 2 presents the ΔH_S°, ΔS_S°, and ΔG_S° for the formation of a specific nucleotide pair next to one already formed. It is seen from the tabulated data that the $\vec{C}\vec{C}$ / $\overset{\leftarrow}{G}\overset{\leftarrow}{G}$ doublet is more stable than $\vec{G}\vec{C}$ / $\overset{\leftarrow}{C}\overset{\leftarrow}{G}$, which is in its turn more stable than $\vec{C}\vec{G}$ / $\overset{\leftarrow}{G}\overset{\leftarrow}{C}$, while the complex consisting of a block of G:C pairs followed by a block of A:U pairs is more stable than the double helix containing repeating A:U and G:C pairs. We may note that it has recently been possible to determine the equilibrium constants for the dimerisation of dideoxyribonucleotides with different sequences by NMR[58]. The sequence of the stabilities of the corresponding minihelices is fully consistent with that predicted by Borer et al.[23]: d(pG–G).d(pC–C) ≥ d(pG–C).d(pG–C) > d(pC–G).d(pC–G). The stabilities of short double helix complexes can be predicted fairly accurately with the aid of the calculated thermodynamic chain propagation parameters and the T_m of polymeric structures with known sequences can be estimated approximately).

It has been observed[59] that the T_m of the double-stranded tetranucleotides C–A–U–G, U–G–C–A, and A–G–C–U, containing equal numbers of the G:C and A:U pairs, are different (25°, 33°, and 34° respectively), i.e. the duplexes with A:U pairs at the ends are more stable than those which terminate in G:C pairs. It has been suggested[59] that a central core of G:C pairs leads to an increase of stability.

One of the factors which increase the stability of the duplexes is the flanking of the double helix by an unpaired (looped) nucleotide residue[19,59], purine nucleotides leading to a greater stabilisation (by ~ 11°) than pyrimidine nucleotides (by ~ 5°).[59] The location of the additional nucleotide residues (the 3′- or 5′-end) is not very important[59]. The cause of the increased stability of the duplex is not completely clear. One of the explanations[6] consists in the fact that the additional "stacking" with the looped bases stabilises the terminal pairs of the duplex, which would otherwise frequently become uncoupled at temperatures below T_m (see below). If the length of the non-complementary shoulders amounts to several nucleotide residues, then the stability of the oligomeric duplexes actually diminishes. For example, a mixture of two icosanucleotides, capable of forming 10 nucleotide pairs, altogether fails to exhibit a cooperative melting profile[60].

Comparison of the stabilities of a series of seven-unit duplexes (system 13, Table 1) of the deoxyribo-series, the ribo-series, and hybrids with similar compositions and sequences (in oligoribonucleotides T is replaced by U) showed that the duplex $dC(pA)_5pG.rC(pU)_5pG$ with the oligo(dA.rU) sequence is extremely unstable, in contrast to the remaining duplexes[3]. The dA clusters with a length corresponding to ~ 5 pairs apparently assume preferentially the B-DNA conformation as a result of which they cannot form stable DNA–RNA hybrids. According to Martin and Tinoco[53], this facilitates the termination of transcription. The influence of the solvent on the thermodynamics of the $d(G–C)_3$ complex formation has been examined[46]. The addition of primary alcohols, formamide, dimethylformamide, or urea to an aqueous buffer solution of $d(G–C)_3$ destabilises the double helix.

V. KINETICS OF THE FORMATION AND DISSOCIATION OF THE OLIGOMERIC COMPLEXES

The kinetic parameters of the formation or dissociation of nucleotide complexes are usually obtained with the aid of the temperature jump method, which is based on the measurement of the time variation of various physicochemical parameters of the system, for example, ultraviolet absorption, after an abrupt increase of temperature. One then obtains the so-called relaxation curve. A characteristic feature of these curves is the relaxation time τ. In all the intermolecular oligomeric complexes investigated the reaction involving the formation of the complementary pairs is of second order and is characterised by a unique relaxation time (from 10^{-3} to 1 s)[22,61] and the temperature at which the maximum amplitude of the relaxation curve is attained is close to the T_m of the duplex[21].

The relaxation time, characterising the complex formation reaction of the oligomers, is related directly to the rate constants for the formation (k_r) and dissociation (k_d) of the double helix and the equilibrium concentrations of the complementary chains (c_A and c_B)[13,22]:

$1/\tau = 4k_r c_A + k_d$ (complex formation by self-complementary oligonucleotides: $A \underset{k_d}{\overset{k_r}{\rightleftharpoons}} AA$);

$1/\tau = k_r(c_A + c_B) + k_d$ (complex formation by non-identical oligomers: $A + B \underset{k_d}{\overset{k_r}{\rightleftharpoons}} AB$).

The rate constants for the forward and reverse reactions can be estimated by plotting $1/\tau$ against the concentration of the free chains.

The complex formation by oligonucleotides can be regarded as a process involving the successive formation of complementary pairs. Since the formation of the nuclei of a nucleotide pair (or several pairs) is a second-order reaction, this stage is a more complex and slower process than the subsequent growth of the double helix[62]. If the formation of the first nucleotide pair is the rate-limiting stage in the complex formation process, then the enthalpy of activation for the latter (ΔH_r^*) should be identical with that of the elementary reaction whose rate is limited by the diffusion of the components: +2 – 6 kcal mol^{-1}.[22] However, the ΔH_r^* for oligo(A) in an acid medium[41], for oligo(A).oligo(U)[61], and oligo(rA).oligo(dT)[13] are negative. In order to resolve this contradiction, Eigen and Pörschke[41] suggested that the stage limiting the rate of the recombination process is the formation of a stable core made up of several nucleotide pairs. The formation of a stable nucleus of the helix is preceded by a stage involving pre-equilibrium between the rapidly formed and decomposed unstable nucleotide pairs. Further growth of the double helix is very rapid. The overall recombination rate constant is then independent of the length of the oligomer chain[13]. On the basis of the analysis of the ΔH_r^*, it was established that, for the complexes containing only the A:U(T) pairs, the core of the helix incorporates ~ 3 pairs of bases[13,41,61,62].

Since the association constant of the G:C pairs is ~ 15 times greater than for the A:U(T) pairs, the recombination of oligonucleotides of mixed composition begins with the formation of the G:C pairs[22]. In these systems k_r changes from 10^5 to 10^7 litre mol^{-1} s^{-1}.[21,22,25,51]. For the $(A)_n.(U)_n.[(T)_n]$ helices, k_r is of the same order of magnitude (10^6 litre mol^{-1} s^{-1}).[61] Having investigated the kinetics of complex formation in heterogeneous systems, Pörschke et al.[22] isolated three characteristic kinetic features: (1) in the given series of oligomers the rate of recombination diminishes rapidly with increase of chain length; (2) the rate of recombination diminishes rapidly with increase of chain length; (2) the rate of recombination of oligomers with identical chain lengths is 6 times faster when the G:C pairs are at the end of the helix and not in its centre; (3) the enthalpy of activation for the formation of oligomeric duplexes containing G:C pairs is positive.

According to the authors[22], the first two features can be explained by the different accessibility of the G:C pairs initiating recombination in oligomeric systems with different nucleotide sequences and lengths. The positive enthalpy of activation for the complex formation by heterogeneous oligomers[21,22] is associated with the fact that the rate-limiting stage in the recombination process is the formation of one or possibly two G:C pairs. In this case there is no need to employ a model incorporating a stage with pre-equilibrium between several pairs of bases.

The dissociation of oligomeric duplexes is much slower than their formation[21,22,25,51,61]. In contrast to k_r, the rate constant for the dissociation of the duplex depends greatly on the length of the helix, i.e. on the number of nucleotide pairs which must be ruptured before the critical intermediate helix is attained[13,22]. The activation enthalpy for the dissociation process ΔH_d^* increases with increasing chain length[13,62].

VI. COMPLEMENTATION INTERACTIONS OF DINUCLEOTIDES

A characteristic property of double helical oligonucleotides is the dependence of the stability of the complex on the chain length and the nucleotide concentration. The relation between T_m, N, and c is given by the equation of Applequist and Damle (see above). The shorter the oligomers, the higher must be their concentration in order to observe the formation of the complementary complex. Thus, at an overall nucleotide concentration of 10^{-4} M, the oligomers capable of complementation interactions must comprise at least several mononucleotide units[10,14]. An increase of nucleotide concentration by a factor of 10 promotes the formation of complexes of tetramers[10]. In connection with the development of the high-resolution NMR technique, in recent years it has proved possible to observe in an aqueous medium the interactions between complementary dinucleotides constituting the smallest structural units containing the 3′ → 5′ phosphodiester linkages. It has been shown[58,63,64] that, in aqueous solutions at high nucleotide concentrations (up to 0.1 M), the self-complementary dinucleoside monophosphates of the ribo-series, namely CpG and GpC[63], and their deoxyanalogues[58], as well as the dinucleotides d(pC–G) and d(pG–C)[58,64] form hydrogen bond–stabilised dimers at low temperatures. The formation of complementary pairs was also observed in concentrated solutions of the CpU + ApC,[53] d(pG–G) + d(pC–C), and d(pG–T) + d(pA–C)[58] mixtures. It is noteworthy that, even at the level of dinucleotide complexes, the dependence of the thermal stability of the miniature double helices on the content of G:C pairs is manifested. Thus a less stable complex is formed in the GpU + ApC mixture than the complex arising in concentrated GpC (CpG) solutions as a result of the self association of the dinucleoside monophosphates, while in the UpA solution and also in the crystalline state (see below) complex formation is not observed at all[63].

VII. THE STRUCTURE OF THE OLIGOMERIC COMPLEXES

1. X-Ray Diffraction Analysis

The most exhaustive information about the steric structure of the double helix oligonucleotides can be obtained by their crystallographic investigation. Single crystals of the following self-complementary oligonucleotides have now been investigated by high-resolution (< 2 Å) X-ray diffraction analysis, which makes it possible to trace individual atoms: UpA[65,66], ApU[67], GpC[68,69], d(pA–T–A–T)[70], and d(C–G–C–G–C–G)[71]. The d(C–G–C–G)[72,73], d(C–G–C–G–A–A–T–T–C–G–C–G)[74,75], and d(G–G–T–A–T–A–C–C)[76] single crystals have been studied with a somewhat smaller resolution (2.25–2.7 Å). In addition, the X-ray diffraction patterns of yet another dimer, namely TpdA, were obtained in 1976.[77] However, instead of single crystals, "oriented" specimens of TpdA, in which the TpdA molecules were built into helical structures (the limit of the resolution in the X-ray diffraction of fibres usually does not exceed 3–4 Å), were investigated.

Among the self-complementary dimers enumerated, only ApU and GpC form Watson–Crick pairs in the crystalline state. The crystal structure of UpA models the loop-like sections of RNA as a result of the specific conformation of the phosphodiester linkage, while TpdA forms unique left-handed helical structures in which the adenine and thymine residues of neighbouring molecules are linked by hydrogen bonds in the "Hoogsteen" manner. We shall dwell in greater detail on the description by Rich and coworkers[67,69] of the crystal structures of ApU and GpC, because theirs were the first data on the Watson–Crick pairs with atomic resolution. Two ApU (or GpC) molecules form in the crystal a segment of the right-handed RNA double helix with the antiparallel directions of their chains. The complementary bases do not lie precisely in a single plane but are slightly tilted. In each chain the bases are arranged in a stack, overlapping to the maximum extent. Such complete "stacking" is a consequence of the purine–pyrimidine sequence of dinucleoside phosphates. The distance between the C(1′) atom of the ribose residues in the A:U pairs is somewhat shorter than the analogous distance in the G:C pairs. This means that the double-helical skeleton is not a completely regular structure but one with systematic "variations", which depend on the nature of the bases. Finally the sites where the counterions are bound depend on the composition and sequence of the bases. The binding of Na^+ ions alters the steric and electrostatic characteristics of the double helix in the sections containing the ApU sequence.

d(pA–T–A–T)[70] in the crystalline state forms a segment of a right-handed double helix with the antiparallel directions of the chains and two pairs of bases stabilised by hydrogen bonds between the first A and the second T in one molecule and the fourth T and the third A in another. The mononucleotide units not involved in the complementation interaction are remote from one another in space. A characteristic feature of the steric structure of d(pA–T–A–T) is different conformations of deoxyribose [C(3′)-*endo* for A and C(2′)-*endo* for T] and different angles of rotation about the glycosidic linkages for the thymidine and adenine residues. The conformations of the phosphodiester linkage in the ...ApT... and ...TpA... fragments also differ. These data served as the starting point for the description of the unusual conformation and hence the special biological role of the poly[d(A–T)].poly[d(A–T)] sections of DNA. Klug et al.[78] suggested that in solution poly[d(A–T)].poly[d(A–T)] assumes the so-called alternating *B* form. In contrast to the classical *B* form of DNA, the repeating structural motif in this double helix consists of two nucleotide pairs.

The most striking results, leading to the discovery of the left-handed double helix of DNA, were obtained by Rich's group in an X-ray diffraction study of d(C–G–C–G–C–G) crystals (resolution 0.9 Å; crystallisation under the conditions of a high ionic strength)[71]. The repeating structural element in this helix, containing 12 G:C pairs per turn, consists of two nucleotide pairs. The alternating guanosine and cytidine residues have different conformations of the glycosidic linkage and of the furanose ring: *syn*- and C(3′)-*endo* for G and *anti*- and C(2′)-*endo* for C. The configuration of

the carbon–phosphate skeleton is very unusual: the phosphate groups are arranged in a zig-zag. This led the authors[71] to call the new structure the *Z*-DNA. Soon the left-handed *Z*-form was discovered also in d(C–G–C–G) on crystallisation at a high ionic strength)[72] and in the poly[d(G–C)].poly[d(G–C)] fibres[79,80]. In their study of the conformational variability of d(C–G–C–G) in the crystalline state, Drew and Dickerson[73] concluded that the *Z*-DNA has a greater conformational flexibility than the *B* form. They described the structural transition from *Z* to *Z'*, associated with a change in the conformation of the furanose ring of guanosine [C(3')-*endo* → C(1')-*exo*] and also the $Z_I \rightarrow Z_{II}$ transition characterising the change in the orientation of the phosphodiester linkage in the ...GpC... unit. The authors conclude that *Z'*, Z_I, and Z_{II} are conformational variants within the limits of the *Z* form. It is of interest that the dodecamer d(C–G–C–G–A–A–T–T–C–G–C–G) exists in the crystalline state as the *B* form with 10.1 bases per turn of the helix, i.e. the terminal C–G–C–G sections are not twisted into the *Z* helix[74].

In 1981 Dickerson and Drew[75] made a detailed X-ray diffraction study with a nearly atomic resolution of the dodecanucleotide d(C–G–C–G–A–A–T–T–C–G–C–G), which forms in the crystalline state a complete coil of the double helix in the *B* form. As a result of the crystal packing effect, the axis of the helix of this duplex is somewhat bent and deviates from the vertical by 19°. It was demonstrated rigorously for the first time that the local parameters of the double helix depend on the sequence of bases, deviating from the classical *B* form, the influence of the nucleotide sequence on the structure of the duplex being much more significant than the influence of the crystal packing and other external factors. Although the local changes in the conformation of the duplex take place within the limits of the *B* form, sections of the double helix whose geometry is more characteristic of the *A* or *D* forms of DNA have been observed. It has been found that purine–pyrimidine steps open their base planes toward the major groove [of the double helix], pyrimidine–purine steps open toward the minor groove"; the bases in the complementary pairs are twisted like the propeller blades and to a greater extent in the A:T pairs. The authors[75,81] reached the important conclusion on the basis of the correlation between the degree of exposure of the sections of the carbohydrate–phosphate skeleton and the rate of cleavage of these sections by the enzyme DNase I, namely that the structure of the dodecanucleotide duplex is identical in both the crystalline state and in solution.

In the next communication of this series[82] the hydrate shell of d(C–G–C–G–A–A–T–T–C–G–C–G) in the crystal was described. It was found that 72 water molecules are arranged around each molecule of the duplex, 50 of which are within the grooves of the helix or in the first coordination shell of the phosphate groups. An attempt was made to estimate the contribution of this factor to the stability of the double helix. The electrostatic potential and the steric accessibility of the reactive sites of the self-complementary dodecamer were calculated from X-ray diffraction data[83]. It was found that a higher negative potential than at the ends is concentrated in the centre of the oligonucleotide duplex. This may be a common feature of such short helices, which is independent of their nucleotide sequence.

The possibility of a wide variety of conformations of the oligonucleotide duplexes of the deoxy-series has been demonstrated[76] in a crystallographic study of d(G–G–T–A–T–A–C–C) containing the fragment of the promoter region of the eukaryotes. It was found that this octamer forms a right-handed double helix in the *A* form. This unusual fact was observed for the first time for deoxyribonucleotide duplexes containing the G:C and A:T pairs. The existence of an oligomer with this sequence in the stable *A* form can be logically related to the functions of the promoter region.

The advances in the X-ray diffraction study of single crystals of self-complementary oligonucleotides open a new page in the study of the fine structure and conformational possibilities of nucleic acids. There is no doubt that the number of complexes investigated by this method will continuously grow, because information about the structural characteristics of nucleic acids is extremely important at the present stage for the elucidation at the molecular level of the mechanism of the protein–nucleic acid recognition and the regulation of gene activity, and mutagenesis.

2. Ultraviolet Spectroscopy and CD(ORD)

Optical methods, namely ultraviolet spectroscopy and CD(ORD), have found extensive applications in the study of the complementation interactions of oligonucleotides in solution. The stoichiometry of the bonding in oligomeric systems is determined with the aid of these methods[10,20]. The formation of a duplex is inferred from the change in the form of the CD spectra of the complementary (self-complementary) oligonucleotides[18,29,84]. Sometimes, in order to achieve more appreciable changes in CD, a ligand which interacts selectively with a double-helical structure, for example distamycin A, is added[84]. The study of the temperature variation of the ultraviolet absorption and the CD(ORD) parameters make it possible to determine T_m and to calculate the thermodynamic parameters of the formation of complementary pairs (Table 1). The change in the ultraviolet absorption of oligonucleotides during the formation (dissociation) of the double helix constitutes the basis of the determination of the kinetic characteristics of the complex formation[13,21]. Furthermore, the ultraviolet spectroscopic and CD(ORD) methods yield useful information about the secondary structure of oligomeric duplexes.

The structural aspects have been traced in a study by Pohl[85] of the dependence of the hypochromicity of the complexes formed by $d(pG–C)_n$ on the number of base pairs in the double helix. Analysis of this relation enabled the author to conclude that the geometrical parameters of the oligomeric duplexes and natural DNA are similar.

More complete information about the structure of the oligomeric complexes can be obtained with the aid of CD. As a consequence of the high sensitivity of the CD to the relative positions of the heterocyclic residues and the conformation of the monomeric units, the similarity of the CD spectra indicates the similarity of the geometrical parameters of the structures being compared. Thus the structural analogy of the triple-stranded $(Ap)_5A.2(Up)_5U$ and oligo(A).2poly(U) helices has been demonstrated with the aid of this method[9].

Comparison of the CD of the oligomeric complexes and natural nucleic acids of known structure is of particular interest. It was found that the CD spectra of complexes of oligoribonucleotides with one another or with oligodeoxyribonucleotides are of the non-conservative type characteristic of the *A* form of RNA in aqueous medium[10,53,86], while the CD spectra of the oligodeoxyribonucleotide complexes are similar to those of native DNA in the *B* form[53,87]. The CD spectra of the complexes formed by $d(pG–C)_n$ in solutions at a low ionic strength[69] and $d(pG–G–T).d(pA–C–C)_{2-4}$[18,89] are similar to those of DNA in the *B* form with a high content of the G:C pairs, while the CD spectrum of $d(pA)_3.d(pT)_{12}$[89] resembles that of DNA containing ~ 30% of G:C pairs. The CD method makes it possible to distinguish readily the different forms of oligomeric double helices in solution. The CD

spectrum of $d(C-G)_3$ in aqueous solution with a low NaCl concentration[32] resembles the spectrum of poly[d(C–G)]. .poly[d(C–G)] recorded under the same conditions and corresponding to the *B* form[90]. The addition of NaCl (up to 5 M) inverts the sign of the long-wavelength Cotton effect, which is associated with the transition of the oligonucleotide duplex to the left-handed *Z* form[32] [the existence of this form for $d(C-G)_n$ under the conditions of high ionic strength has been demonstrated by X-ray diffraction]. According to CD data, the *Z* form of $d(C-G)_2$ is unstable[32]. In the case of $d(A-T)_3(C-G)_3$, which forms a concatameric complex in which the $d(C-G)_n$ block is flanked by an alternating A–T sequence, the transition to the Z form is not observed according to CD data[32] [cf. the self-complementary d(C–G–C–G–A–A–T–T–C–G–C–G), which has been investigated by X-ray diffraction—see above].

The CD spectra have been calculated recently for numerous possible conformations of d(T–G–G–C–G–T–A–T–T–C–T). .d(A–G–A–A–T–A–C–G–C–C–A) and it has been shown that the CD amplitude at 275 nm varies linearly with the helix winding angle and the base pair twist[91]. The establishment of this correlation is important for the study of the dependence of the CD of nucleic acids on structural parameters.

According to modern ideas, the CD spectra of nucleic acids depend not only on the secondary molecular structure but also on the nucleotide sequence. Gray and Tinoco[92] developed a theory, according to which the form of the CD spectrum of DNA is determined by the interaction of mainly the neighbouring nucleotide pairs. Evidently the dependence of CD on the base sequence should be manifested particularly clearly at the level of oligomeric duplexes, because, in contrast to DNA, in the latter the frequencies of the encounters of specific combinations of two nucleotide pairs are not averaged out. Fig. 3 presents as an example the CD spectra of isomeric ribonucleotide duplexes[86]. The oligomeric duplexes with known sequences have been used in two investigations[86,93] to test the possibility of the determination of the nucleotide sequence of nucleic acids with the aid of CD. The contributions of all 10 combinations of two Watson–Crick nucleotide pairs have been isolated[86] on the basis of the analysis of the CD spectra of a series of oligonucleotide duplexes. The spectra obtained for the isomeric dinucleotide dimers differ sharply both in form and in amplitude. The CD spectra of the polymeric helices, constructed taking into account the spectroscopic contributions isolated, are in poor agreement with experimental curves. Hillen et al.[93] attempted to calculate the CD spectra of 12 restriction fragments of DNA with lengths ranging from 12 to 360 nucleotide pairs. For this purpose, use was made of a set of spectroscopic contributions of specific combinations of two nucleotide pairs taken from the spectra of synthetic double-helical polynucleotide (homogeneous or with repeating di- and trinucleotide sequences). The calculated and experimental spectra agree satisfactorily, with the exception of the positive CD band at 270 nm, which is particularly sensitive to conformational changes. The discrepancy is particularly notable for duplexes containing less than 43 nucleotide pairs apparently as a result of end effects.

3. Nuclear Magnetic Resonance

Together with the optical methods examined, NMR has been widely used recently in the study of the oligomeric complexes[24–27,39,46,52,58,59,87,94–106]. This method permits a detailed description of the steric organisation of the complexes in solution, the elucidation of the dynamics of the dissociation of the double-helical structures, and the estimation of the degree of structural analogy between the oligomeric duplexes and natural DNA and RNA. The overall effects accompanying the helix–coil transition are recorded with the aid of optical methods, while NMR makes it possible to trace the behaviour of individual base pairs.

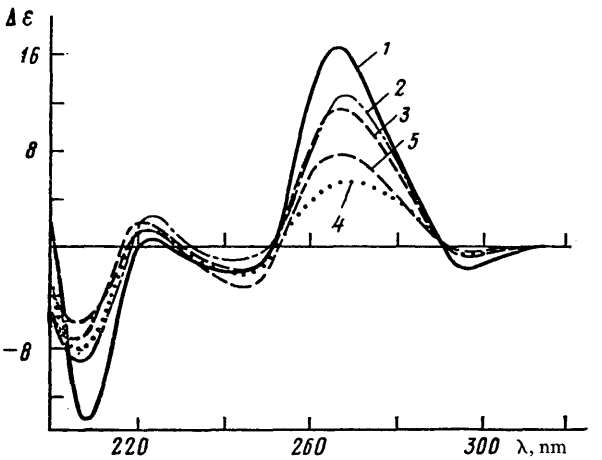


Figure 3. CD spectra of five double helical isomeric complexes containing four A:U and two G:C base pairs (1 M NaCl, pH 7.0, $c = 10^{-2}$ M per monomer unit): 1) A_2GCU_2; 2) A_2CGU_2; 3) U_2CGA_2; 4) $A_4G_2 + C_2U_4$; 5) $A_4CG + CGU_4$.[86]

It has now become possible to resolve and assign on high-class instruments (270, 300, 360, 500 and 600 MHz) the majority of the resonance signals of the protons of the bases and the sugar rings in double-helical oligonucleotides containing up to 12 pairs of bases and to reach a conclusion concerning the local environment of each proton. This made it possible to observe independently the changes in the character of the environment of each nucleotide pair of the duplex as the concentration, temperature, pH, etc. are varied. In the case of long nucleotide duplexes, the broadening of the resonance lines is associated with the rigidity of the polymeric nucleic acids, which prevents the rapid movement of the segments of the nucleotide chains in solution. The ^1H NMR spectra of a series of such duplexes, namely $A_{25}.U_{25}$,[107] $d(C_{15}A_{15}).d(T_{15}G_{25})$,[108] $dG_n.rC_{22}$, $dG_n.rC_{11}dC_{16}$, and $dG_n.dC_{22}$ ($n > 70$)[109], as well as two restriction fragments of DNA containing 43 and 69 base pairs[102], have been obtained, but the proton signals of the individual bases have not been assigned.

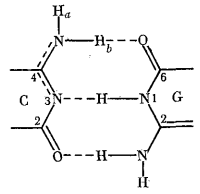
The ^{31}P NMR (145.7 MHz) spectra of oligonucleotide duplexes have been obtained recently[52,98]. They contain data concerning the conformation of the carbohydrate–phosphate skeleton of the oligomers forming the double helix. The breakdown of the "stacking" of the bases at temperatures exceeding the T_m of the duplex has been detected with their aid. In 1980 Cohen[103] analysed the ^{31}P NMR spectra of oligo d(G–C) obtained previously and suggested that the presence of two well resolved ^{31}P signals in 4 M LiCl and of a single signal at a low concentration of the salt indicate a left-handed *Z* form of the helix in these oligomers in a concentrated salt solution.

Two different groups of proton signals are investigated in order to obtain information about the structure of the oligomeric complexes.

(1) The signals of the non-exchanging protons linked to the carbon atoms of the heterocyclic bases or the sugar—the doublets of the H(5) and H(6) protons of the pyrimidine bases [the H(6) singlet in the case of thymine], the singlets of the H(2) and H(8) protons of the purine bases, the singlet of the CH_3 protons of thymine, and the signals of the protons in the sugar ring, mainly H(1'). They are all located in the range 4–9 p.p.m. relative to sodium 4,4-dimethyl-4-silapentanesulphonate (DSS). The spectra are investigated in D_2O or in buffer solutions containing D_2O. The positions of the oligonucleotide signals are determined as a rule by the standard "incremental assignment" method[26,27,110,111] by measuring the δ for the dimers, trimers, and other fragments of the given oligomer and assuming that the values of δ do not in the main change on passing to the intact oligomer. The chemical shift δ of the signal of each proton is subjected to the shielding influence of the neighbouring bases and also the bases in the complementary chain. Furthermore, the position of the signal is determined by the conformation of the furanose ring, the rotation of the base around the glycosidic linkage, the anisotropy of the phosphate group, and the interchain hydrogen bonds. Consequently δ depends on the structure of the double helix. On thermal dissociation of the complex, the signals of the non-exchanging protons of the bases shift both downfield and upfield[27]. Curves for the temperature dependence of the chemical shifts of the signals of the non-exchanging protons permit a direct determination of the thermodynamic characteristics of the helix–coil transition. Here one can trace the dissociation of each nucleotide pair of the duplex, although the assignment and measurement of the changes in the signals as a function of temperature remain a difficult problem.

(2) The signals of slowly exchanging ring protons of the imino-groups N(1)–H of guanine and N(3)–H of uracil (thymine), which are directly involved in the formation of hydrogen bonds between the bases (Watson–Crick pairs). The spectra are recorded in H_2O or in aqueous buffer solutions. The N(1)–H resonance signal of guanine characterises the G:C pair and the N(3)–H signal of U (T) characterises the A:U(T) pair. We may note that the resonances of the imino-group protons of the free oligomers are not observed in water owing to the rapid exchange between the protons with the solvent. The formation of a hydrogen bond leads to a slower exchange of the protons with water and to the appearance of resonance signals in the region of weak fields (12–15 p.p.m. relative to DSS). The presence of such signals in the ^{1}H NMR spectrum of short oligonucleotides is convincing evidence for the formation of a duplex. With increase of temperature, an upfield shift and a broadening and disappearance of the signals, associated with the increase of the relative content of the open-chain forms and the rapid exchange of the imino-group protons in the open-chain form with water, are as a rule observed. The temperature of the disappearance of the signal is not related to the thermodynamic stability of the double helix and yields only comparative characteristics of the stabilities of different nucleotide pairs of the duplex, because the resonance signals of the N(1)–H protons of G and the N(3)–H protons of T (U) are markedly broadened even at a concentration of the open-chain forms of ~ 10% and cannot be traced further. The observation of the behaviour of the individual signals on gradual increase of temperature helps in their assignment[27,52,99,112]. Here account is taken of the fact that the resonance of the proton of the imino-group of the "isolated" G:C pair is observed in a stronger field (13.6 p.p.m.) than the corresponding resonance of the A:T pair (14.6 p.p.m.).[96] A procedure involving chemical modification is sometimes used in the assignment[99] or the calculated and experimental chemical shifts are compared[27]. When triply-stranded oligomeric complexes are formed, the signals of the imino-group protons corresponding to the formation of "reverse" Hoogsteen A:U pairs are observed[27,104] in addition to the signals of the exchanging protons of the Watson–Crick pairs in the range 14.6–15 p.p.m.

The signals of the exchanging protons of the imino-groups of the C(4)–NH_2 base of cytosine and the C(2)–NH_2 base of guanine, located in the range 6–9 p.p.m. relative to DSS, are considered together with the protons of the imino-group. The exchange of the amino-group protons with the solvent is slower than that of the imino-group protons[98]. The partial double bond character of the C–NH_2 linkage in cytosine restricts the rotation of the amino-group and therefore at low temperatures individual broad signals are observed for each of the protons of the NH_2 groups of cytosine. In complementation interactions the signal of the proton involved in the formation of a hydrogen bond (H_b in the scheme below) shifts downfield, whereas the signal of the proton accessible to the solvent (H_a) undergoes an upfield shift. For guanine, a single resonance signal due to the two protons of the amino-group is observed, which is explained by the rapid rotation of the latter about the C–NH_2 bond.



On formation of the complementary pair, the average signal of the NH_2 protons of guanine shifts downfield. The dependence of the chemical shift of this signal on the nucleotide concentration can be used to estimate the equilibrium complex formation constant for the dinucleotides[58,63]. Furthermore, by analysing the temperature dependence of the width of the proton signals of the amino-groups in cytosine and guanine, it is possible to discover if the rotation of the amino-group about the C–NH_2 bond in the oligomeric duplexes is rapid or slow[98]. It is noteworthy that these signals are more often used to detect the formation of complementary complexes between dinucleoside phosphates (dinucleotides)[58,63,64].

Detailed ^{1}H NMR study of the structural characteristics of a number of oligomeric systems showed that complexes containing the d(A–T–G–C–A–T)[96,113], d(pG–C)$_4$, d(pT–A)$_{3,4}$, d(pA–G)$_4$.d(pC–T)$_4$,[112] d(A–A–A–G–C–T–T–T)[114], d(C–C–G–G), d(G–G–C–C)[98], d(G–G–A–A–T–T–C–C)[52], d(A–T–C–C–T–A–T–A–G–G–A–T)[99], d(C–T$_5$–G).d(C–A$_5$–G)[27], and d(A–A–T–T–G–C–A–A–T–T)[106] deoxyribonucleotide units assume in solution a conformation close to that of the *B* form of DNA, while the geometry of the *A*(*A'*) form of RNA is characteristic of the complexes of the ribo-derivatives A–A–G–C–U–U[26,97], C–C–G–G[96], and A–G–C–U[105] as well as the d(C–T$_5$–G).r(C–A$_5$–G) hybrid derivatives[27]. This conclusion was reached as a result of a comparison of the calculated and experimental chemical shifts for the exchanging and non-exchanging protons of the bases of a given nucleotide pair of the oligomeric complex. In the calculation of the chemical shift, the influence of the magnetic fields of the ring currents on the signals of the corresponding protons was determined (without taking into account the influence of

other factors) for all possible combinations of the neighbouring base pairs, taking into account the known geometrical parameters of the *A*, *B*, and *D* forms of DNA[112,113,115]. One should bear in mind that agreement between the calculated and experimental chemical shifts of the signals of the exchanging protons is by no means always observed[27] and frequently only for the inner pairs of bases in the duplexes[95,96,98,112,114]. Signals of the protons of the end pairs are in a stronger field than required by the calculation. According to a number of investigators[95,112,114], equilibrium between the hydrogen-bonded and open forms are characteristic of the end nucleotide pairs of the duplexes (the signals of the open form were manifested in a stronger field) and the chemical shift of the signals of the imino-group protons is determined by the ratio of the populations of the two forms. An alternative explanation of the upfield shift of the signals of the exchanging protons in the terminal nucleotide pairs is the formation of intermolecular structures as a result of the "stacking" of the ends of the oligomeric duplexes[98]. A discrepancy between the calculated and experimental values of δ has been noted also for the non-exchanging protons of the terminal pairs and has been attributed to the stacking interaction of the ends of the duplexes[52,98]. In a study of the double helical A–G–C–U and A–C–G–U, the δ for the non-exchanging protons of the bases were calculated much more rigorously[105]. The calculated and experimental δ for the majority of the non-exchanging base protons of A–C–G–U did not agree, in contrast to its isomer. The authors concluded that the double helix in A–C–G–U is a non-standard form, and emphasised the importance of this conclusion for the estimation of the conformational possibilities in the RNA duplexes.

The conformation of the furanose rings in the nucleotide units of the complex, which determines the form of the double helix, has also been established with the aid of ^{1}H NMR data. For this purpose, measurements were made of the spin–spin vicinal coupling constants of the protons at C(1′) and C(2′) and were compared with the values predicted for different conformations of furanose. It was found that, for all the carbohydrate residues in d(A–T–G–C–A–T)[113], d(C–C–G–G), and d(G–G–C–C)[98], under complex formation conditions, the C(3′)-*exo*-conformation is characteristic of DNA in the *B* form. The two deoxyribose residue in the d(C–T_5–G).d(C–A_5–G) duplex, belonging to d(C–A_5–G), are 90% in the C(2′)-*endo*-conformation and the double helix therefore has the geometry of the *B* form[27]. In contrast to these systems, the furanose rings of all the monomeric units of the complex formed by A–A–G–C–U–U[26] or all the monomeric units of the ribo-chain of the d(C–T_5–G).r(C–A_5–G) complex[27] are in the C(3′)-*endo*-conformation characteristic of RNA in the *A*(*A*′) form[26]. Analysis of the temperature variation of $J_{1',2'}$ led to the conclusion that the C(3′)-*endo*-conformation of the furanose rings predominates, so that the helices in the duplexes formed by C–A–U–G[111] and A–G–C–U[105] and also in G–A–G–C.G–C–U–C[110] have the *A*(*A*′) form.

Additional information about the secondary structure of the oligomeric complexes was obtained in the study of the interaction with the antibiotic actinomycin D of the double helices enriched in G:C pairs. A characteristic property of this antibiotic is its specific binding to DNA in the *B* form and its lack of affinity for RNA and hybrid helices[116]. It was shown by ^{1}H NMR that the phenoxazinone ring of actinomycin D is inserted between the G:C pairs of the double helical oligodeoxyribonucleotides[96,112,117,118], which indicates a DNA-like conformation of such complexes in aqueous solution.

The formation of the A–G–C–U or A–C–G–U duplexes[105] entails a change (by 20° and more) of the glycosidic angle of twist in the adenosine and cytidine residues. This was demonstrated by analysing the δ for the anomeric H(1′) protons.

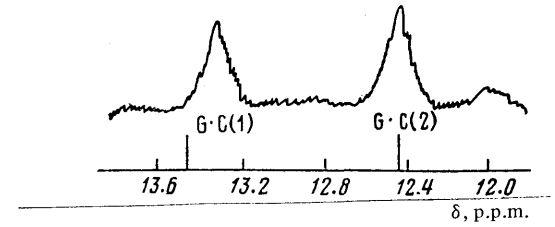


Figure 4. NMR spectrum (220 MHz) of the exchanging imino-group protons of the guanine residues in the duplex

CpCpGpG
GpGpCpC
1 2 3 4

at 0 °C in water. The calculated positions of the resonance signals, subject to the condition that the structure of the double helical CpCpGpG corresponds to the structure of RNA-11, are indicated by lines[95].

The study of the temperature variation of the chemical shifts and the signal widths of the exchanging protons of the oligomeric duplexes led Patel and coworkers[52,94,117] to the conclusion that, under the conditions of the thermodynamic stability of the helix, there is a probability of the incomplete bonding of the terminal nucleotide pairs in such systems (regardless of the nucleotide composition and sequence). In other words, the rapid formation and rupture of the hydrogen bonds between the bases of the terminal nucleotide is postulated, the equilibrium shifting towards an increase of the relative content of the open forms with increase of temperature. The rapid formation and dissociation of nucleotide pairs at the ends of the double helix ("fraying") is much faster and occurs at a lower temperature than the thermodynamic fusion–separation of the strands of the duplex, and leads to an average resonance signal whose chemical shift and width are determined by the ratio of the open forms and the forms stabilised by hydrogen bonds. Fig. 4 presents the ^{1}H NMR spectrum of the duplex formed by C–C–G–G at 0 °C. The calculated positions of the proton signals of the terminal and inner G:C pairs are indicated by lines. If it is assumed that the upfield shift and the broadening of the signals due to the protons of the terminal G:C pairs are due to the increase of the relative content of the open forms, it is found that at 0 °C the terminal base pairs C–C–G–G.C–C–G–G are "fused" to the extent of 8%.[95] The analogous analysis of the ^{1}H NMR spectrum of the duplex formed by d(A–T–G–C–A–T) leads to the conclusion that, under the conditions of the stability of the double helix, the bases of the A:T end pairs are in the open form to the extent of 15%.[96] With decrease of temperature, the signals of the imino-group protons of the A:T and T:A pairs of the double helical d(A–T–G–C–A–T) undergo a downfield shift; the displacement of the signals of the protons of the terminal A:T pairs of the duplex is observed down to −30 °C (in aqueous methanol) and that of the signals of the inner T:A pairs is observed down to −20 °C. Patel and Hilbers[94] interpreted this as an increase of the fraction of the hydrogen-bonded forms with decrease

of temperature. According to their calculations, at 3 °C the fraction of the open forms is 22.5% for the terminal A:T pairs and 1.1% for the inner T:A pairs, being absent for the central G:C pairs. With increase of temperature, the reverse process, namely a successive increase of the relative content of the open forms (initially the terminal and then the inner nucleotide pairs undergo ring opening) is observed for oligonucleotide complexes of any composition and with any sequence[27,94,95,99,112]. Fig. 5 presents the ^{1}H NMR spectra of the complex formed by A–A–G–C–U–U in the region of a weak field at different temperatures. At 1 °C the absorption lines of the imino-group protons are observed at 13.2, 13.5, and 14.2 p.p.m. (each signal corresponds to two identical resonances of the imino-group protons of equivalent nucleotide pairs). With increase of temperature, all three signals are broadened and disappear in the following sequence: initially the signal at 13.2 p.p.m., to which correspond the terminal A:U pairs, then the signal at 14.2 p.p.m. associated with the inner A:U pairs, and finally the signal at 13.5 p.p.m. associated with the central G:C pairs[97]. Thus the signals of the less stable (owing to the incomplete pairing) terminal A:U pairs are broadened and disappear faster than the signals of the inner A:U and G:C pairs shielded from the effect of the solvent. In the d(A–T–C–C–T–A–T–A–G–G–A–T) duplex fusion also begins from the ends of the double helix. There is no preliminary fusion of the central A–T fragment, i.e. the sequence of four A:T pairs, protected by the G:C pairs, is too short for the local destabilisation of the duplex[99].

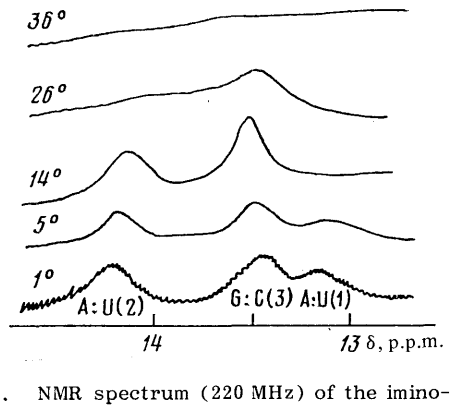


Figure 5. NMR spectrum (220 MHz) of the imino-group protons of the duplex

ApApGpCpUpU
UpUpCpGpApA
1 2 3 4 5 6

at different temperatures in H_2O (1 M NaCl, pH 7.6).[97]

In the study of the temperature variation of δ of the non-exchanging protons, the T_m of individual nucleotide pairs of duplexes were determined and it was shown that they depend on the composition and sequence of the bases. On dissociation of the double helical oligonucleotides without terminal G:C pairs, namely d(A–T–G–C–A–T)[51] and A–A–G–C–U–U,[26] the stability of the nucleotide pairs increases in the following sequence: terminal A:U(T) pairs < inner A:U(T) pairs < central G:C pairs. The tendency of the terminal pairs to undergo "differential" melting (i.e. before the melting of the remainder of the duplex) is greatly reduced for the duplexes in which the A:U pairs are internal and the more stable G:C pairs are terminal. Thus stepwise dissociation is not observed (the helix–coil transition takes place in accordance with the "all or nothing" principle[27]) for the C–A–U–G[111], G–A–G–C.G–C–U–C[110], d(G–G–A–A–T–T–C–C),[52] and d(C–A_5–G).d(C–T_5–G)[27] duplexes, as well as C–C–G–G which is made up exclusively of G:C pairs[95] and the T_m of the individual nucleotide pairs, determined with the aid of ^{1}H NMR (of the non-exchanging protons), corresponds to the T_m of the helix obtained by optical methods. On the other hand, the above tendency increases sharply for the d(pT–A)$_3$ duplex: at 5 °C the nucleotide chains are retained mainly via two central A:T pairs[100]. The result of studies of the A–G–G–A.U–C–C–U and U–G–C–A duplexes whose terminal A:U pairs melted at the same temperature as the inner G:C pairs[59], are in some conflict with these data. For the same nucleotide composition and the same number of inner G:C pairs in the A–G–C–U and U–G–C–A duplexes, the terminal residues are dissociated in the former but not in the latter[59]. Finally, the dissociation of the terminal pairs diminishes in the presence of unpaired (looped) bases[59] (see above). This information is useful for the understanding of the nature of the RNA–RNA interactions in the cell. In the study of the duplexes formed by the self-complementary d(G–G–C–C) and d(C–C–G–G), Patel observed a difference between the temperature variations of δ of the non-exchanging protons of the inner and outer G:C pairs[98]. He believes that these differences are due to "end to end" aggregation of the duplexes resulting from the stacking interactions of the end pairs and leading to a greater shielding of the protons of these pairs. As a result, the signals of the protons of the terminal G:C pairs are observed in stronger fields. With decrease of temperature, they continue to shift upfield (without reaching a plateau) and the T_m determined with their aid is smaller than the T_m for the inner pairs. It is noteworthy that the quantitative aspects of the analysis of the temperature variations must be approached with caution, since the T_m determined from the temperature variations of the signals of different non-exchanging protons within the limits of the same nucleotide residue sometimes differ appreciably[7]. The error in the determination of T_m is smaller for the duplexes formed by non-self-complementary oligonucleotides, since in this case one can take into account the temperature variation of the protons of each chain[27]. In the description of the "differential" fusion of the terminal nucleotide pairs of oligomeric duplexes, some workers unjustifiably used the term "fraying" of the ends[59,100,110], which reflects a kinetic effect (see above) and is in no way related to the equilibrium concentration of the open and hydrogen-bonded forms[27]. For example "fraying" of the ends occurs in d(C–T_5–G).d(C–A_5–G), but the duplex does not decompose and the content of the partly fused forms is very low[27].

A study[99] of the duplex d(A–T–C–C–T–A–T–T–A–G–G–A–T), containing one non-complementary T:T pair, provides a good illustration of the possibilities of the NMR method. It was shown that the tridecanucleotide forms a regular structure similar to DNA in the *B* form and that the non-canonical T:T pair does not emerge from the helix. However, it is of interest that the incorporation of the T:T pair diminishes appreciably the overall stability of the double helix as a result of the formation of an additional unstable region where fusion begins as well as in the terminal sections, as can be seen from the disappearance of the signals of the exchanging protons.

The NMR study of the relaxation behaviour of the resonances of the exchanging protons of the bases of an oligomeric duplex, namely the 12-unit restriction fragment d(C–C–G–C–A–C–T–G–A–T–G–G).d(C–C–A–T–C–A–G–T–G–C–G–G), was carried out recently for the first time[101]. Analysis of the rates of relaxation and of their temperature variation made it possible to examine a completely new aspect of the behaviour of the duplexes—the conformational fluctuations of the helix. The absence of internal dynamics in the 12-unit DNA fragment, at least on the 10–100 ns time scale, was observed[101]. It was shown that there is no reorientation of the bases (relative to the axis of the helix) and that this fragment can be regarded as a rigid rod. Furthermore, a remarkable observation was made concerning the mechanism of the opening of the individual base pairs. It was found that the exchange of the imino-group protons of the thymine residues with the solvent at 30–40 °C is not accompanied by the opening of the neighbouring G:C pairs. On this basis, the authors[101] concluded that the rates of the random opening of the A:T pairs in any DNA are independent of the nucleotide sequence and length and are therefore identical for all types of DNA. This conclusion has been confirmed experimentally in the study of the restriction fragments of DNA consisting of 43 and 69 units[102].

In a number of ^{1}H NMR studies of the complex formation by oligomers, detailed investigations were made of the kinetic aspects of the exchange of the imino-group protons with water[24,25,97]. These data made it possible to estimate the lifetime of the base pairs in the helical complex and to obtain independently the kinetic parameters of the initiation and propagation of the oligomeric double helix[25]. The calculated activation energies and rate constants for nucleation and dissociation agree well with the corresponding data obtained for ribo-oligonucleotides with the aid of the temperature jump method (Section V). The exchange process responsible for the broadening of the resonance lines can be described as follows in general terms[24,114]:

$$NH_h \underset{k_{ch}}{\overset{k_{hc}}{\rightleftarrows}} NH_c \xrightarrow{k_{H_2O}} H_2O \ .$$

According to this scheme, the imino-group protons can exist in one of three states: within the double helical structure (NH_h), in the open form (NH_c), and as part of the H_2O molecule. The equilibrium constant of the transition from the hydrogen-bonded (h) to the free (c) state is $K_{eq} = k_{hc}/k_{ch}$. The characteristics of these exchange processes are now well known[119]. Equations describing the relations between the widths and positions of the resonance lines of the double-stranded complex and the rates of the exchange reactions have been derived[52,97,119]. Further details of the mechanism of the exchange of the imino-group protons with water have been reported[24,119].

4. Column Chromatography

Oligonucleotides immobilised on a carrier can form complexes with complementary homogeneous[120–122] and heterogeneous[121,123] oligonucleotides (including the formation of *hybrid ribodeoxyribonucleotide complexes*[120,121]). Cellulose[120,121,123], hexamethylenediamine-Sepharose[123] to which the oligonucleotide was linked covalently, and DEAE-cellulose, in which the irreversible sorption of the oligonucleotide linked covalently to a soluble polymer [poly(vinyl alcohol)] has taken place[122], are used as carriers. Under temperature gradient conditions, the complementary oligonucleotide is desorbed and the stability of the complex can be estimated approximately from the temperature at which the peak emerges. It has been shown that all the bases of the immobilised oligomer participate in the formation of hydrogen bonds[120,121] and that this process is highly specific: a single non-complementary pair is sufficient to prevent the sorption of the oligonucleotide[124]. Chromatography on carriers with oligonucleotides is discussed in the literature mainly as a method for the separation of oligonucleotide mixtures[120–123].

REFERENCES

1. D.Riesner and R.Römer, in "Physico-chemical Properties of Nucleic Acids" (Edited by J.Duchesne), Acad.Press, London–New York, 1973, **2**, p.237.
2. P.O.P.Ts'o, in "Basic Principles in Nucleic Acid Chemistry", Acad.Press, New York–London, 1974, **2**, p.411.
3. C.R.Cantor and P.R.Schimmel, "Biophysical Chemistry", W.H.Freeman and Co., San Francisco, 1980, Vol.III, p.1183.
4. N.C.Seeman, in "Nucleic Acid Geometry and Dynamics" (Edited by R.H.Sarma), Pergamon Press, New York–Oxford, 1980, p.109.
5. N.I.Sokolova, V.K.Kagramanova, and N.G.Dolinnaya, Uspekhi Khim., 1975, **44**, 104 [Russ.Chem.Rev., 1975, **44**(1)].
6. N.R.Kallenbach and H.M.Q.Berman, Rev.Biophys., 1977, **10**, p.138.
7. D.R.Kearns, Ann.Rev.Biophys.Bioeng., 1977, 6, 477.
8. R.H.Sarma, in "Nucleic Acid Geometry and Dynamics" (Edited by R.H.Sarma), Pergamon Press, New York–Oxford, 1980, p.143.
9. C.R.Cantor and W.W.Chin, Biopolymers, 1968, **6**, 1745.
10. R.B.Gennis and C.R.Cantor, Biochemistry, 1970, 9, 4714.
11. D.Pörschke, Biopolymers, 1971, **10**, 1989.
12. G.R.Cassani and F.J.Bollum, Biochemistry, 1969, **8**, 3928.
13. J.G.Hogget and G.Maass, Ber.Bunsenges.Phys. Chem., 1971, **75**, 45.
14. R.Naylor and P.T.Gilham, Biochemistry, 1966, **5**, 2722.
15. F.M.Pohl, Eur.J.Biochem., 1974, **42**, 495.
16. I.E.Scheffler, E.L.Elson, and R.L.Baldwin, J.Mol.Biol., 1970, **48**, 145.
17. F.N.Hayes, E.H.Lilly, R.L.Ratliff, D.A.Smith, and D.L.Williams, Biopolymers, 1970, 9, 1105.
18. N.G.Dolinnaya, E.S.Gromova, S.N.Mikhailov, and Z.A.Shabarova, Bioorg.Khim., 1978, **4**, 535.
19. F.H.Martin, O.C.Uhlenbeck, and P.Doty, J.Mol. Biol., 1971, **57**, 201.
20. S.R.Jaskunas, C.R.Cantor, and I.Tinoco, Jr., Biochemistry, 1968, **7**, 3164.
21. S.K.Podder, Eur.J.Biochem., 1971, **22**, 467.
22. D.Pörschke, O.C.Uhlenbeck, and F.H.Martin, Biopolymers, 1973, **12**, 1313.
23. P.N.Borer, B.Dengler, and I.Tinoco, Jr., J.Mol.Biol., 1974, **86**, 843.
24. D.M.Crothers, C.W.Hilbers, and R.G.Shulman, Proc.Nat.Acad.Sci. USA, 1973, **70**, 2899.
25. C.W.Hilbers and D.J.Patel, Biochemistry, 1975, **14**, 2656.

26. P.N.Borer, L.S.Kan, and P.O.P.Ts'o, Biochemistry, 1975, **14**, 4847.
27. A.Pardi, F.H.Martin, and I.Tinoco, Jr., Biochemistry, 1981, **20**, 3986.
28. V.N.Dubrynin, V.G.Korobko, I.V.Severtsova, N.S.Bystrov, E.F.Boldyreva, B.K.Chernov, and M.N.Kolosov, Bioorg.Khim., 1978, **4**, 1600.
29. Z.A.Shabarova, N.G.Dolinnaya, S.I.Turkin, and E.S.Gromova, Nucleic Acids Res., 1980, **8**, 2413.
30. Z.A.Shabarova, E.M.Volkov, T.S.Oretskaya, S.I.Turkin, N.G.Dolinnaya, V.K.Kagramanova, and M.A.Prokof'ev, Dokl.Akad.Nauk SSSR, 1981, **258**, 914.
31. Z.A.Shabarova, N.G.Dolinnaya, V.L.Drutsa, N.P.Melnikova, and A.A.Purmal, Nucleic Acids Res., 1981, **9**, 5747.
32. F.Quadrifoglio, G.Manzini, M.Vasser, K.Dinkelspiel, and R.Crea, Nucleic Acids Res., 1981, **9**, 2195.
33. M.T.Doel, M.A.W.Eaton, E.A.Cook, H.Lewis, T.Patel, and N.H.Carey, Nucleic Acids Res., 1980, **8**, 4575.
34. N.K.Kochetkov, E.I.Budovskii, E.D.Sverdlov, N.A.Simukova, M.F.Turchinskii, and V.N.Shibaev, in "Organicheskaya Khimiya Nukleinovykh Kislot" (Organic Chemistry of Nucleic Acids), Izd.Khimiya, Moscow, 1970, p.216.
35. B.H.Zimm, J.Chem.Phys., 1960, **33**, 1349.
36. D.Poland and H.A.Scheraga, "Theory of Helix-Coil Transitions in Biopolymers", Acad.Press., New York–London, 1970.
37. J.Applequist and V.Damle, J.Amer.Chem.Soc., 1965, **87**, 1450.
38. J.Gralla and D.M.Crothers, J.Mol.Biol., 1973, **78**, 301.
39. D.Albergo De Prisco, L.A.Marky, K.J.Breslauer, and D.H.Turner, Biochemistry, 1981, **20**, 1409.
40. K.J.Breslauer, J.M.Sturtevant, and I.Tinoco, J.Mol.Biol., 1975, **99**, 549.
41. M.Eigen and D.Pörschke, J.Mol.Biol., 1970, **53**, 123.
42. J.Applequist and V.Damle, J.Amer.Chem.Soc., 1966, **88**, 3895.
43. P.O.P.Ts'o, N.S.Kondo, M.P.Schweizer, and D.H.Hollis, Biochemistry, 1969, **8**, 997.
44. D.W.Appleby and N.R.Kallenbach, Biochemistry, 1973, **12**, 2093.
45. A.Adler, L.Grossman, and G.D.Fasman, Proc.Nat. Acad.Sci.USA, 1967, **57**, 423.
46. D.Albergo De Prisco and D.H.Turner, Biochemistry, 1981, **20**, 1413.
47. M.M.Warshaw and C.R.Cantor, Biopolymers, 1970, **9**, 1079.
48. L.Kotin, J.Mol.Biol., 1963, **7**, 309.
49. O.C.Uhlenbeck, F.N.Martin, and P.Doty, J.Mol.Biol., 1971, **57**, 217.
50. T.E.England and T.Neilson, Canad.J.Biochem., 1977, **55**, 365.
51. D.J.Patel, Biochemistry, 1975, **14**, 3984.
52. D.J.Patel and L.L.Canel, Eur.J.Biochem., 1979, **96**, 267.
53. F.H.Martin and I.Tinoco, Jr., Nucleic Acids Res., 1980, **8**, 2295.
54. J.Marmur and P.Doty, Nature, 1959, **186**, 1427.
55. R.D.Wells, J.E.Larson, R.C.Grant, B.E.Shorile, and C.R.Cantor, J.Mol.Biol., 1970, **54**, 465.
56. M.Karstadt and J.S.Krakow, J.Biol.Chem., 1970, **245**, 752.
57. J.Gralla and D.M.Crothers, J.Mol.Biol., 1973, **73**, 497.
58. M.A.Young and T.R.Krugh, Biochemistry, 1975, **14**, 4841.
59. T.Nielson, P.J.Romaniuk, D.Alkema, D.W.Hughes, J.R.Everett, and R.A.Bell, Nucleic Acids Res., Symposium Series, No.7, 1980, p.293.
60. N.K.Gupta, E.Ohtsuka, V.Sgaramella, H.Büchi, A.Kumar, H.Weber, and H.G.Khorana, Proc.Nat. Acad.Sci.USA, 1968, **60**, 1341.
61. D.Pörschke and M.Eigen, J.Mol.Biol., 1971, **62**, 361.
62. M.E.Craig, D.M.Crothers, and P.Doty, J.Mol. Biol., 1971, **62**, 383.
63. T.R.Krugh, J.W.Laing, and M.A.Young, Biochemistry, 1976, **15**, 1224.
64. T.R.Krugh and M.A.Young, Biochem.Biophys. Res.Communs, 1975, **62**, 1025.
65. J.Rubin, T.Brennan, and M.Sundaralingam, Biochemistry, 1972, **11**, 3112.
66. J.L.Sussman, N.C.Seeman, S.-H.Kim, and H.M.Berman, J.Mol.Biol., 1972, **66**, 403.
67. N.C.Seeman, J.M.Rosenberg, F.L.Suddath, J.J.P.Kim, and A.Rich, J.Mol.Biol., 1976, **104**, 109.
68. B.Hingerty, E.Subramanian, S.D.Stellman S.B.Broyde, T.Sato, and R.Langridge, Biopolymers, 1975, **14**, 227.
69. J.M.Rosenberg, N.C.Seeman, R.O.Day, and A.Rich, J.Mol.Biol., 1976, **104**, 145.
70. M.A.Viswamitra, O.Kennard, P.G.Jones, G.M.Sheldrick, S.Salisbury, and L.Falvello, Nature, 1978, **273**, 687.
71. A.H.J.Wang, G.J.Quigley, F.J.Kolpak, J.L.Crawford, J.H.van Boom, G.van der Marel, and A.Rich, Nature, 1979, **282**, 680.
72. H.Drew, T.Takano, S.Tanaka, K.Itakura, and R.E.Dickerson, Nature, 1980, **286**, 567.
73. H.R.Drew and R.E.Dickerson, J.Mol.Biol., 1981, **152**, 723.
74. R.Wing, H.Drew, T.Takano, C.Broka, S.Tanaka, K.Itakura, and R.E.Dickerson, Nature, 1980, **287**, 755.
75. R.E.Dickerson and H.R.Drew, J.Mol.Biol., 1981, **149**, 761.
76. Z.Shakked, D.Rabinovich, W.B.T.Kruse, E.Egert, O.Kennard, G.Sala, S.A.Salisbury, and M.A.Viswamitra, Proc.Roy.Soc. B, 1981, **213**, 479.
77. H.R.Wilson and J.Al-Mukhtar, Nature, 1976, **263**, 171.
78. A.Klug, M.A.Viswamitra, O.Kennard, Z.Shakked, and T.A.Steitz, J.Mol.Biol., 1979, **131**, 669.
79. S.Arnott, R.Chandrasekaran, D.L.Birdsall, A.G.W.Leslie, and R.L.Ratliff, Nature, 1980, **283**, 743.
80. G.Gupta, M.Bansal, and V.Sasisekharan, Biochem. Biophys.Res.Comm., 1980, **95**, 728.
81. G.P.Lomonossoff, P.J.G.Butler, and A.Klug, J.Mol.Biol., 1981, **149**, 745.
82. H.R.Drew and R.E.Dickerson, J.Mol.Biol., 1981, **151**, 535.
83. R.Lavery and B.Pullman, Nucleic Acids Res., 1981, **9**, 3765.
84. R.Weiss, E.Birch-Hirschfeld, C.Zimmer, and G.Luck, Nucleic Acids Res., 1979, **6**, 757.
85. F.M.Pohl, FEBS Letters, 1974, **38**, 202.
86. P.N.Borer, O.C.Uhlenbeck, B.Dengler, and I.Tinoco, Jr., J.Mol.Biol., 1973, **80**, 759.

87. P.S.Miller, D.M.Cheng, N.Dreon, K.Jayaraman, L.S.Kan, E.E.Leutzinger, S.M.Pulford, and P.O.P.Ts'o, Biochemistry, 1980, **19**, 4688.
88. F.M.Pohl and T.M.Govin, J.Mol.Biol., 1972, **67**, 375.
89. N.G.Dolinnaya, E.S.Gromova, E.V.Il'ina, N.F.Sergeeva, Z.A.Shabarova, and M.A.Prokof'ev, Bioorg.Khim., 1975, **1**, 1296.
90. F.M.Pohl and T.M.Jovin, J.Mol.Biol., 1972, **67**, 375.
91. B.B.Johnson, K.S.Dahl, I.Tinoco, Jr., V.I.Ivanov, and V.B.Zhurkin, Biochemistry, 1981, **20**, 73.
92. D.M.Gray and I.Tinoco, Jr., Biopolymers, 1970, **9**, 223.
93. W.Hillen, T.Goodman, and R.D.Wells, Nucleic Acids Res., 1981, **9**, 3029.
94. D.J.Patel and C.W.Hilbers, Biochemistry, 1975, 14, 2651.
95. D.B.Arter, G.C.Walker, O.C.Uhlenbeck, and P.G.Schmidt, Biochem.Biophys.Res.Comm., 1974, **61**, 1089.
96. D.J.Patel and A.E.Tonelli, Proc.Nat.Acad.Sci.USA, 1974, **71**, 1945.
97. L.S.Kan, P.N.Borer, and P.O.P.Ts'o, Biochemistry, 1975, **14**, 4864.
98. D.J.Patel, Biopolymers, 1977, **16**, 1635.
99. A.G.Cornelis, J.H.J.Haasnoot, J.F.Hartog, M.Rooij, J.H.van Boom, and A.Cornelis, Nature, 1979, **281**, 235.
100. D.R.Phillips and G.C.K.Roberts, Biochemistry, 1980, **19**, 4795.
101. T.A.Early, D.R.Kearns, W.Hillen, and R.D.Wells, Biochemistry, 1981, **20**, 3756.
102. T.A.Early, D.R.Kearns, W.Hillen, and R.D.Wells, Biochemistry, 1981, 20, 3764.
103. J.S.Cohen, Trends in Biochem.Sci., 1980, 5, 58.
104. H.A.Geerdes, and C.W.Hilbers, Nucleic Acids Res., 1977, **4**, 207.
105. E.Bubienko, M.A.Uniack, and P.N.Borer, Biochemistry, 1981, **20**, 6987.
106. G.Lancelot, M.Roger, N.T.Thuong, M.Chassignot, and C.Hélène, Biochimie, 1981, **63**, 785.
107. M.J.Heller, A.T.Tu, and G.E.Maciel, Biochemistry, 1974, **13**, 1623.
108. T.A.Early, D.R.Kearns, J.F.Burd, G.E.Lascon, and R.D.Wells, Biochemistry, 1977, **16**, 541.
109. E.Selsing, R.D.Wells, T.A.Early, and D.R.Kearns, Nature, 1978, **275**, 249.
110. D.W.Hughes, R.A.Bell, T.E.England, and T.Neilson, Canad.J.Chem., 1978, **56**, 2243.
111. P.J.Romaniuk, T.Neilson, D.W.Hughes, and R.A.Bell, Canad.J.Chem., 1978, **56**, 2249.
112. D.J.Patel and A.E.Tonelli, Biopolymers, 1974, **13**, 1943.
113. D.J.Patel and A.E.Tonelli, Biopolymers, 1975, **14**, 3990.
114. N.R.Kallenbach, W.E.Daniel, and M.A.Kaminker, Biopolymers, 1976, **15**, 1218.
115. C.Geissner-Prettre and B.Pullman, J.Theor.Biol. 1970, **27**, 87.
116. H.M.Sobell and S.C.Jain, J.Mol.Biol., 1972, **68**, 21.
117. D.J.Patel, Biochemistry, 1974, **13**, 2396.
118. A.D.Cross and D.M.Crothers, Biochemistry, 1971, **10**, 4015.
119. D.M.Crothers, P.E.Cole, C.W.Hilbers, and R.G.Shulman, J.Mol.Biol., 1974, **87**, 63.
120. C.R.Astell and M.Smith, Biochemistry, 1972, 11, 4114.
121. C.R.Astell, M.T.Doel, P.A.Jahnke, and M.Smith, Biochemistry, 1973, **12**, 5068.
122. H.Schott, H.Eckstein, and E.Bayer, J.Chromat., 1974, **99**, 31.
123. E.V.Il'ina, V.D.Smirnov, Z.A.Shabarova, and M.A.Prokof'ev, Bioorg.Khim., 1976, **2**, 376.
124. H.Schott, FEBS Letters, 1974, **41**, 203.

Faculty of Chemistry,
Lomonosov Moscow State University